BACTERIAL AND FUNGAL PROFILE OF ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment of the regulations

for the award of the degree of

M.D.(MICROBIOLOGY)

BRANCH – IV

MADRAS MEDICAL COLLEGE

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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APRIL 2015

CERTIFICATE

This is to certify that this dissertation titled **"BACTERIAL AND FUNGAL PROFILE OF ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE** " is a bonafide record of work done by **DR.V.R.YAMUNADEVI**, during the period of her Post Graduate study from MAY 2012 to APRIL 2015 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of **M.D MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R Medical University to be held in April 2015.

Dr.R. VIMALA M.D
Dean
Madras Medical College &
Government General Hospital,
Chennai – 600 003.

Dr.G. JAYALAKSHMI,M.D.,DTCD,
Director,
Institute of Microbiology,
Madras Medical College&
Government General Hospital
Chennai – 600 003.

DECLARATION

I declare that the dissertation entitled **"BACTERIAL AND FUNGAL PROFILE OF ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE** "submitted by me for the degree of M.D. is the record work carried out by me during the period of October 2013 to September 2014 under the guidance of **Dr. Sheila Doris, M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical Unversity, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2015.

Place : Chennai
Date:

Signature of the candidate
(Dr. V.R.YAMUNADEVI)

Signature of Guide,
Prof.Dr.T.SHEILA DORIS.,MD,
Professor,
Institute of Microbiology,
Madras Medical College, Chennai-03

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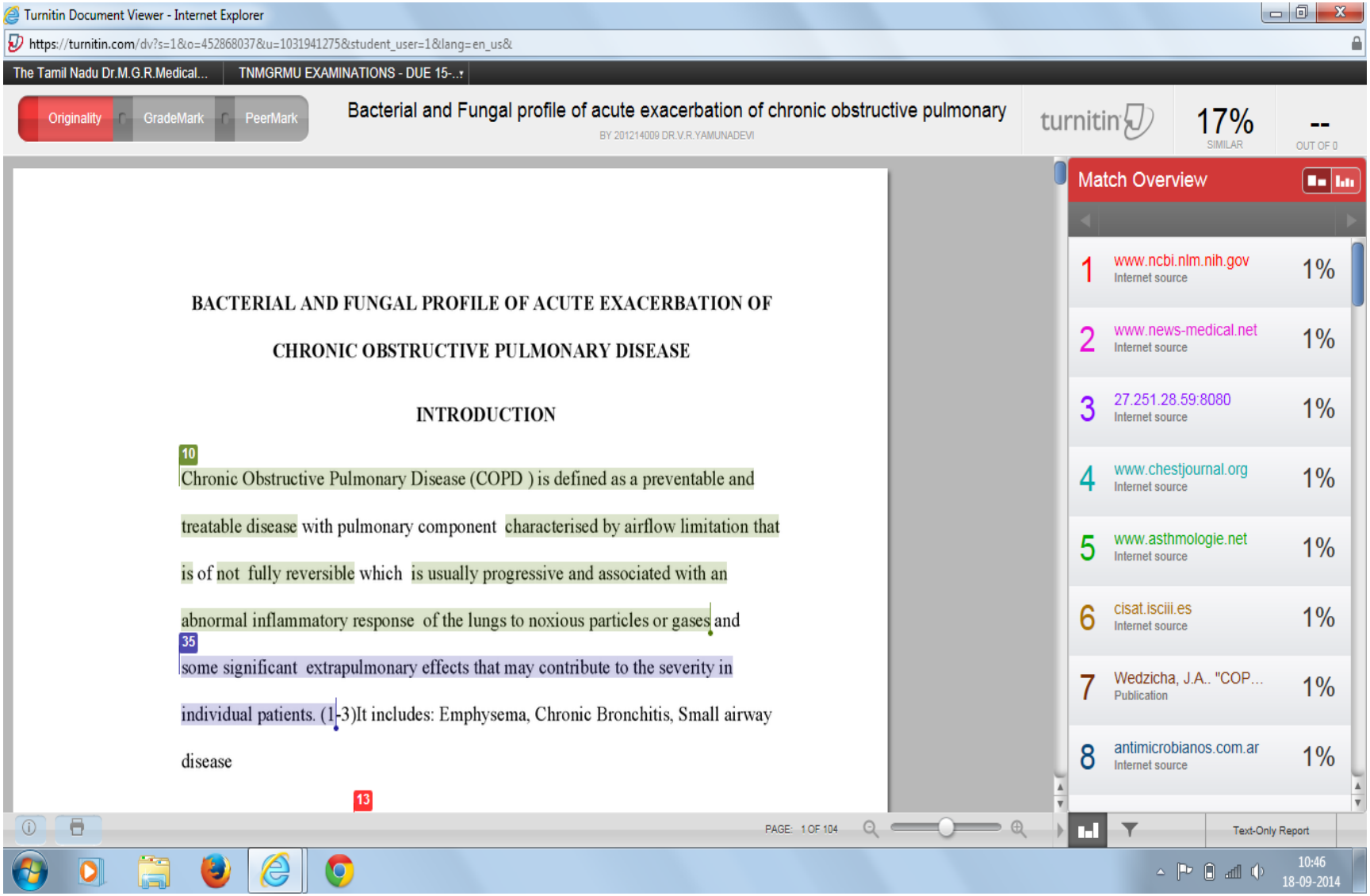
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individual patients. (1-3)It includes: Emphysema, Chronic Bronchitis, Small airway
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ABSTRACT

BACKGROUND AND OBJECTIVE: Acute exacerbation of Chronic obstructive Pulmonary disease (AECOPD) is defined as a sustained worsening of the patient's condition ,from the stable state in the patient's baseline dyspnoea and cough or sputum ,or both and beyond normal day to day variation ,that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD as per Gold guidelines .It leads to significant increase in morbidity and mortality in COPD patients. Bacteria are responsible for 60% of exacerbation. The aim of our study was to determine the bacterial and fungal isolates in AECOPD and Stable COPD patients with special reference to antibiotic susceptibility and their resistance pattern from hospital data.

SETTINGS AND DESIGN : It was a Cross sectional study carried out at the Institute of Microbiology, Madras Medical College in association with Departments of Internal Medicine, Thoracic Medicine, Intensive Medicare Care Unit at Rajiv Gandhi Government General Hospital, Chennai from October 2013 to September 2014

MATERIALS AND METHODS : The Study population consisted of 150 in patients presenting with signs and symptom of AECOPD and 50 stable COPD out patients .All the respiratory samples were subjected to direct gram staining , culture ,biochemical reactions and the isolates were identified ac-cording to standard techniques. Antibiotic sensitivity was done by Kirby-Bauer method according to CLSI standards.

RESULTS : COPD was common in age group of sixty to seventy one. Both AECOPD and Stable COPD patients had male predominance .Tobacco smoking was strongly associated with study group. Respiratory failure 8% was the most common complication .Positive bacteriological culture was obtained in 70.6% of cases. Mixed infection among AECOPD patients was found in 3.8% of culture positive cases. Sputum purulence was significantly correlated with the culture positivity . The commonest organism in the respiratory samples in AECOPD patients were Gram negative bacteria 74.5% as compared to Gram positive

bacteria 21.6.Among Gram negative organisms *Klebsiella pneumoniae* 33.3% was the most commonly and significantly isolated organism followed by *Pseudomonas aeruginosa* .In stable COPD patients only 19% *Klebsiella pneumoniae* was isolated. Non fermenters were significantly isolated in Severe type and *Staphylococcus aureus* from Moderate type. *Klebsiella pneumoniae* showed Multi drug resistance(MDR) of 29.7% The prevalence of Carbapenemase production in *Klebsiella pneumoniae* was 28.5%This implies that *Klebsiella pneumoniae* was one of the important drug resistant pathogen isolated among AECOPD patients. Presence of MRSA and ESBL isolates were higher in Moderate AECOPD patients (66.6%, 66.6% , respectively) than in severe group(33.3%,33.3%). Penicillin resistant *Streptococcus pneumoniae* , Imipenem resistance, Carbapenamase producing isolates,Amp C producing strain were significantly higher in severe AECOPD patients MDR pathogens were present both in moderate and severe type of COPD.
Haemophilus influenzae and Fungus were not isolated.

CONCLUSION : The bacterial etiology of AECOPD is different in India from what has been shown in western studies..To conclude Gram negative bacteria were more frequently isolated in our patients, antimicrobial treatment should be started early depending on the antimicrobial sensitivity results, in the wake of an increasing rate of isolation of resistant organisms.

BACTERIAL AND FUNGAL PROFILE OF ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is defined as a preventable and treatable disease with pulmonary component characterised by airflow limitation that is of not fully reversible which is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases and some significant extrapulmonary effects that may contribute to the severity in individual patients [1-3]. It includes: Emphysema, Chronic Bronchitis, Small airway disease

AECOPD(acute exacerbation of COPD): This condition is defined as a sustained worsening of the patient's condition ,from the stable state(in the patient's baseline dyspnoea and cough or sputum ,or both and beyond normal day to day variation ,that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD as per Gold guidelines [4]. It is characterized by presence of increased sputum volume, sputum purulence and dyspnoea.

Burden of the disease : Chronic obstructive pulmonary disease (COPD) is associated with significant morbidity and mortality, with the World Health Organization estimating its rise from being the fourth to the third leading cause of death by 2030. The mortality rates are supposed to increase by 30% every decade[5].

Almost 95% of mortality due to chronic respiratory disease in India can be assigned to COPD.

Exacerbations of COPD have considerable impact on health care system at both primary and tertiary care levels as they are the major reason for antibiotic use and admissions. WHO has estimated that 600 million people worldwide have COPD. Additionally, exacerbations lead to indirect costs because of days lost from work. COPD affects 30% of patients seen in chest clinics and constitutes 1-25% of hospital admissions all over India[6].

AE-COPD is a common cause of emergency room (ER) visits and is a major cause of morbidity and mortality [7.8].

A gross underestimate of COPD Prevalence had been estimated as 17 million and it is likely to increase by over 30% in next decade. Highest prevalence (9.4%) was reported from North Indian rural population from a study conducted by Jindal et.el from 1964-1995.

***Causes of AE-COPD*:**

Exacerbations are caused or triggered by a variety of factors including viruses, bacteria ,and air pollutants, and are associated with acutely increased worsening of existing (acute-on-chronic) airway inflammation and also due to defects in host defence mechanisms. Alterations produced in the bronchial epithelium by the damaging action of smoking favour bacterial adhesion and colonization. In turn, airway colonization and chronic infection contribute to progressive pulmonary

damage via the action of proinflammatory substances in what is known as the "vicious circle theory[9].

Infections are the important cause of acute exacerbation. Bacteria are responsible for causing 60% of exacerbations. Viral infections are the likely cause of approximately 30% of exacerbations, while PCR studies have suggested that up to 40% of acute respiratory infections in COPD are associated with viruses. Fungal isolates have not been reported[3,10].

Exacerbations, mostly of an infectious etiology, are a frequent cause of morbidity in COPD patients. Furthermore, infection was the most common observable cause of death in prospectively followed-up COPD patients[11].

This condition is highly serious in our country as the prevalence of smoking and air pollution is very high which are the main cause for COPD and increase the frequency of exacerbation. Little has been documented about this problem from India.

Exacerbations punctuate the clinical course of COPD in many patients. Since it is a vicious cycle recurrent exacerbation will lead to rapid deterioration of lung parameters and early death due to respiratory failure and increased economic burden. These episodes of acute exacerbation can vary considerably in severity as part of the exacerbations will remain unreported while some episodes require admission. A European survey found that sputum analysis of exacerbated patients is requested only in 10% of cases [11].

Antimicrobial therapy: Over 90% of patients with AECOPD are treated with antibiotics, on empirical basis without proper sputum analysis so the effectiveness of treatment is uncertain due to emerging new strains and their resistant pattern thereby leading to recurrent exacerbation[113]. It would be useful to find the proper etiology of COPD exacerbations, thereby facilitating the orientation of antibiotic treatment and reducing the high number of failures recorded with empiric treatment, which in some cases, is as high as 26%[114].

This study is taken up to find out the Bacteriological & Fungal profile and their sensitivity pattern in AECOPD and Stable COPD patients as the knowledge of possible bacterial & fungal etiology and sensitivity patterns of COPD exacerbations, facilitates the orientation of antibacterial and antifungal treatment so that timely institution of correct management is important for better prognosis of disease and to improve the quality of life of the patient.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORICAL REVIEW:

The term exacerbation has its origin in the Latin descriptive *acerbus*, meaning harsh, bitter, sharp and more at outer edge.

Hippocrates (460-377BC): The Father of Medicine described an old men suffering from breathlessness associated with cough and catarrhal

Earliest references to COPD

In 1679 Bonet described Chronic Obstructive Pulmonary Disease (COPD) as "voluminous lungs". It was corroborated around a century later in 1769 by Morgagni who described cases in which the lungs were "turgid", particularly from air.

Baillie in 1789 published a series of illustrations of the emphysematous lung putting forth the pathology of the disease. Thus emphysema was known to be a part of COPD.

It was much later that chronic bronchitis got included in COPD.

Badham in 1814 used the word catarrh to refer to the chronic cough and increased mucus secretion as symptoms of bronchiolitis and chronic bronchitis that could be part of COPD.

Laënnec described emphysema of the lungs in 1821 in his Treatise of diseases of the chest. He was the inventor of the stethoscope who wrote that emphysema lungs

were excessively inflated that did not empty well. Laënnec went on to describe a combination of emphysema and chronic bronchitis. Our present knowledge of the disease is founded on the clinical work of Laennec.

In 1855 Bierner was given the credit for studying the sputum in Bronchopulmonary disease.

In 1846 John Hutchinson invented the spirometer. This was the key to diagnosing COPD. The spirometer is still used today for diagnosis and regular assessment regarding response to therapy in COPD. Hutchinson's instrument only measured vital capacity.

In 1915 Dass and Luetscher studied and described the application of bacteriological sputum examination and recognised Haemophilus influenza as a common cause of acute and chronic Bronchitis

In 1947 Tiffeneau and Pinelli added the concept of timed vital capacity as a measure of airflow.

In 1964 Eriksson showed that people with a severe congenital deficiency of serum α1 antitrypsin developed Emphysema.

William Briscoe is believed to be first person to use the term COPD in discussion at the 9th Aspen Emphysema conference. This term became established and today we refer to COPD as the designation of this growing health problem.[9,12]

DEFINITION :

Chronic Obstructive Pulmonary Disease (COPD) is defined as a preventable and treatable disease with pulmonary component characterised by airflow limitation that is of not fully reversible which is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases and some significant extrapulmonary effects that may contribute to the severity in individual patients. [1,2]

It was also defined in a joint statement of American Thoracic Society and the European Respiratory Society as a disease characterised by and diagnosed with spirometric measurement of airflow limitation that is not fully reversible which is also supported by GOLD.[13]

COPD includes Emphysema, Chronic Bronchitis, Small airway disease.

Emphysema : It is defined as an abnormal ,permanent enlargement of the distal airspaces ,distal to the terminal bronchioles ,accompanied by destruction of their walls and without obvious fibrosis.

Chronic Bronchitis: A clinically defined condition with presence of chronic productive cough on most days for 3 months in each of 2 consecutive years in a patient in whom other causes of chronic cough have been excluded.[3]

Small airway disease : It is a condition in which small bronchioles are narrowed .

DISEASE CLASSIFICATION:

COPD is a heterogenous disease which has many hypotheses like British, American, Dutch, Swedish and all the hypotheses probably have elements of truth as COPD is a classic gene –by-environment disease.

In newer literature COPD severity is classified as per 2006 revision of GOLD criteria that is based on post bronchodilator lung function

GOLD 1(mild)	$FEV_1/FVC < 0.70$ and $FEV_1 >= 80\%$ predicted
GOLD 2 (moderate)	$FEV_1/FVC < 0.70$ and $80\% > FEV_1 >= 50\%$ predicted
GOLD3(severe)	$FEV_1/FVC < 0.70$ and $50\% > FEV_1 >= 30\%$ predicted
GOLD 4(very severe)	$FEV_1/FVC < 0.70$ and $FEV_1 < 30\%$ predicted or FEV1 < 50% predicted plus chronic respiratory failure or signs of heart failure

People with $FEV_1/FVC >= 0.70$ and respiratory symptoms of chronic cough and sputum production are no longer included as COPD stage (formely GOLD stage 0).Patients with $FEV_1/FVC >= 0.70$ but an FVC <80% predicted meet spirometric criteria for a restrictive process. Although this is not regarded as COPD ,patients might present with several symptoms similar to those seen in COPD ,and these patients have an increased risk of death.[14]

AECOPD(acute exacerbation of COPD): This condition is defined as a sustained worsening of the patient's condition ,from the stable state(in the patient's

baseline dyspnoea and cough or sputum ,or both and beyond normal day to day variation ,that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD as per Gold guidelines [4]

Anthosien criteria :[15]

- Increased breathlessness
- Increased volume of sputum
- Increased purulence of sputum

There are 3 types of acute exacerbations of chronic bronchitis which are based upon 3 cardinal symptoms which include worsening of dyspnea, increase in sputum purulence, and increase in sputum volume. The 3 types are defined as follows:

1. Type 3: Mild exacerbation with 1 of 3 cardinal symptoms PLUS 1 of the following:
    a. Upper respiratory tract infection in the past 5 days
    b. Fever without other apparent cause
    c. Increased wheezing
    d. Increased cough
    e. Increased respiratory rate or heart rate by 20% above baseline

2. Type 2: Moderate exacerbation with 2 of 3 cardinal symptoms

3. Type 1: Severe exacerbation with all 3 cardinal symptoms

Cardinal symptoms include worsening of dyspnea, increase in sputum purulence, and increase in sputum volume .[16]

BURDEN OF DISEASE :

COPD is a growing global epidemic and it is estimated to kill around 3 million people every year .It is currently the 4th largest killer disease in the world and expected to climb to 3rd position by the year 2030 .

WHO has estimated that 600 million people worldwide have COPD. It affects around 5-10% of population over the age of 40 years but still there wide variations in the prevalence between countries.(17)

ECONOMIC BURDEN:

The National Heart , lung and Blood Institute provides a estimate of 38.8 billion US $ for US and 38.6 billion US $ for Europe .COPD accounts for 56% of total health care budget.

According to National Commission on macroeconomics and health published in India that per capital expenditure on COPD is Rs.42664 in 2006 and expected to increase to Rs. 62630 by 2016. Upto 84% of the costs spent on COPD is due to inpatient hospitalization due to which the loss in productivity due to COPD account for between 40% and 67% of the overall costs across the world .Hence it is a severe economic burden for countries throughout the world.(10)

Morbidity: In Canada 1 in 4 people older than 35 years was likely to be diagnosed with COPD. The burden was more in rural men ,with lower socioeconomic status (Eurorespiratory society annual congress in Amsterdam ,Netherlands 2011).

In Canada by about 80 years about 25% of women and about 30% of men will be diagnosed with COPD.[18]

8-22% of adults aged more than 40 years and older is the leading cause of hospitalization and health care cost incurrence [19].It is a common and leading cause of morbidity which is the major cause of worsening of Quality of life.50% of patients who survive first hospitalization get readmitted within 6 months . Estimated prevalence rates for people more than 30 years vary between 0.6% and 4% in men and 0.2 – 32% in women(yin an et al.Passive smoking exposure and risk of COPD among adults in China .[20]

INDIA:

A gross underestimate of COPD Prevalence had been estimated as 17 million and it is likely to increase by over 30% in next decade. Highest prevalence(9.4%) was reported from North Indian rural population from a study conducted by Jindal et.el from 1964-1995[10] .

MORTALITY:

Global mortality: WHO states that more than 3 million people die of COPD per year and 5% of all deaths globally and 160% in South east asian region over the next two decades. India itself contributes to over half of a million deaths second only to China .These mortality rates are supposed to increase by 30% every decade[5]

SOUTH EAST ASIAN REGION :[5]

Almost 5,56,000 deaths are attributable to COPD as estimated by WHO in South east asian region which majorily comprises INDIA .So almost 95% of mortality due to chronic respiratory disease in India can be assigned to COPD. State wise data is at present available for Maharashtra .As the data for other states are not available , studies on COPD is a must to assess the burden.

DALYS (Disability adjusted life year):

In 1990 COPD accounted for 2.1% of DALY'S which ranked 12th most common cause .This is expected to increase upto 4.1% and it is assumed to move to 5th rank by 2020.[20,21].Overall COPD was estimated to have resulted in more than 26 million DALYS in 2000.

In the year 2002 6740 thousand DALYs were lost due to COPD in South East Asian region.

In India COPD account for 3% of DALYS but this is likely to be underestimated .[17]

DEFENCE MECHANISM OF NORMAL LUNG RESIDENT DEFENSES

1. Airway architecture
2. Epithelial barrier
3. Mucociliary clearance
4. Soluble factors in airway secretion –complement,immunoglobulins
5. Alveolar macrophages

Recruited Defenses

If resident defence system is not able to control the Polymorphonuclear neutrophils and lymphocytes are recruited to augment host response by the production of Leukotriene β 4,TNF- α.[22]

RISK FACTORS FOR COPD[106,107]:

1. Smoking-cigarrete or Bidi-50% of smokers develop COPD.73% of mortality in COPD is due to smoking among which 40% is from low and middle socioeconomic status. Smoking leads to ciliary destruction and hypermucus secretion and decreased mucociliary clearance.

2. Aging-As the lung function starts to decline by third and fourth decade of life

3. Tuberculosis (this is very common in India)

4. Respiratory infection in early life

5. Passive or second hand smoking

6. Ambient air pollution-WHO estimates 1% of COPD cases in high income countries is due to urban air pollution where as it is 2% in nations of low and middle income.

7. Occupational exposure-coal mining,cotton textile dust, mining

8. House hold exposure-biomass fuels -Ninety percent of rural households and 32% of urban households cook their meals on a biomass stove.3 billion people are exposed to biomass all over the world and it carries the same amount of

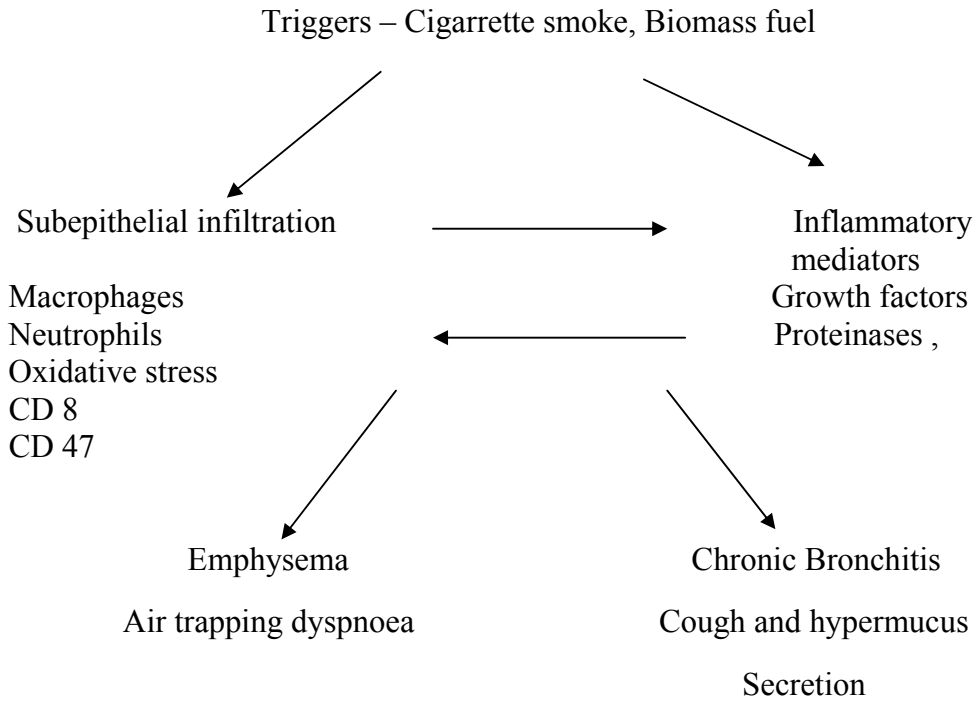
risk of developing COPD as tobacco smoke. WHO states that 35% of population in countries of low and middle income develop COPD due to its inhalation and 36% of mortality from lower respiratory disease is due biomass inhalation.

9. Low Socioeconomic status

10. Genetic factors-α1 antitrypsin deficiency leads to emphysema in 1-3% of patients

11. Gender-In high income countries COPD prevalence is similar in both sexes due to smoking where as condition is different in low and middle income countries as smoking in female is low .[23]

PATHOLOGICAL CHANGE IN COPD:

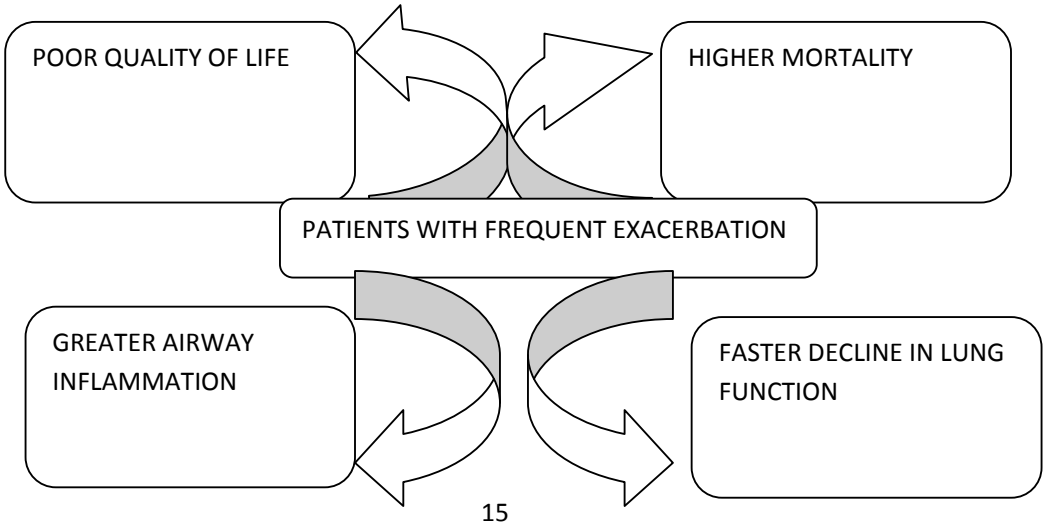


PATHOPHYSIOLOGY OF AECOPD:[3.10]

1. In COPD patients due to defective phagocytosis of macrophages it leads to constant bacterial colonization of respiratory tract and frequent ,recurrent exacerbation of COPD. This can amplify and lead to increase airway and systemic inflammation in Stable COPD patients and this vicious cycle increases progressively with disease severity. As airflow obstruction progresses the frequency of exacerbation also increases.[24,25]As an increase in concentration of bacteria that colonize the lower respiratory tract constitutes to the pathogenesis of exacerbation and the acquisition of new bacterial strain are also crucial in the pathogenesis of exacerbation.[26]

2. Alveolar macrophages from COPD patients phagocytose lesser number of apoptotic epithelial cells .Therefore there is chronic bacterial colonization with Streptococcus pneumonia ,Haemophilus influenzae which attributes to acute infectious exacerbation .[27]

EFFECT OF COPD EXACERBATION WITH FREQUENT EXACERBATION:(Vicious cycle)



CAUSES OF ACUTE EXACERBATION OF COPD:[3,28]

INFECTIOUS AGENTS :

Virus , Gram positive and gram negative aerobic bacterial, atypical bacteria

ENVIRONMENTAL CONDITIONS:

Sudden change in temperature ,humidity,air pollution exposure,tobacco smoke exposure,noxious gases or irritating chemicals

HOST FACTORS:

Patients with poor general health, poor nutrition, immunocompromised state ,lack of compliance to prescribed medicines,adoption of unhealthy life styles modes, poor level of personal hygiene,lack of compliance with long term oxygen therapy, failure to participate in pulmonary rehabilitation.

FACTORS THAT POTENTIALLY MODIFY RISK FACTORS OF AECOPD

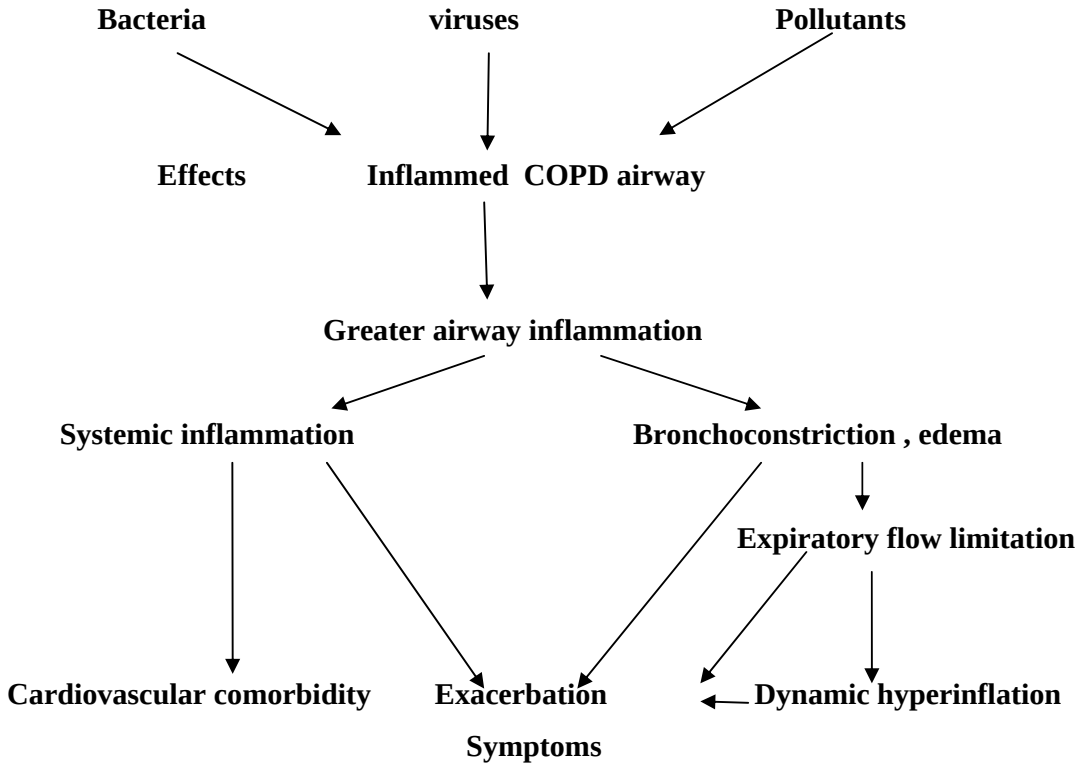
INTRINSIC FACTORS

- Impairement of lung functions
- Active smoking
- Bronchial hyperresponsiveness
- Chronic Mucus secretion
- Impairement of defence mechanism
- Non specific factors: increase age, comorbid illness

EXTRINSIC FACTORS:

- Type of bacterial infection and changing strains of bacteria
- Decreased environmental temperature
- Air pollution
- Type of treatment for Stable and exacerbation of COPD

TRIGGERS OF COPD EXACERBATIONS AND ASSOCIATED PATHOPHYSIOLOGICAL CHANGES LEADING TO INCREASED EXACERBATION SYMPTOMS



Among all causes infection is the most common precipitating factor .Molecular diagnostics have given strong evidence that Microorganisms are involved in 80% of cases but the interaction between microorganisms and host is more important .[3,9,10]

Viral infections:

It accounts for 30% of infection but it could be an underestimate as it is difficult to isolate. The common viruse are *Rhinovirus, Coronavirus, Influenza virus, Parainfluenza virus, Adenovirus, Respiratory syncytial virus*

Bacterial infections:

It accounts for 60% of infection. Most common are *Haemophilus influenzae nontypable, Moraxella Catarrhalis, Streptococcus pneumoniae.*A number of studies have shown that virulent organisms are isolated in severe AECOPD patients like Staphylococcus aureus, *Pseudomonas aeruginosa* and members of Enterobactericeae family.[10]

Atypical bacteria:

The role of *Chlamydia , Legionella and Mycoplasma* is conflicting in causing AECOPD as these microorganisms might also interact with airway bacteria and viruses. A study done by using real time PCR by Diederen .et.al. found no role for these atypical bacteria in AECOPD.[29]

Potter et.al. proved that although a variety of commensal bacteria inhabits nasopharynx of all healthy individuals but still lower airways are usually found to be sterile by standard culture techniques[30].

In Stable COPD:

The precise role of bacteria in causing exacerbation has been difficult to assess as lower airway in stable COPD patients are colonised by same organisms as those

isolated at exacerbations like *Haemophilus influenzae nontypable, Moraxella Catarrhalis, Streptococcus pneumoniae , Pseudomonas aeruginosa* and also consists of other oropharyngeal commensal bacteria like-*Streptococcus viridans, Streptococci,Neisseria sp,Corynebacterium sp,and Candida sp.* A study by Wilkinson TM Donald son et.al.found that bacteria was isolated in 48.2% of stable COPD patients which rose to 70% in AECOPD patients.[31]A study by Berenson .et. al. isolated bacteria in 25% of stable COPD and 50% in AECOPD[32].

Purulent sputum is the surrogate marker of bacterial infection since isolation rate of bacteria is more in purulent sputum as compared to mucoid sputum.

Since in this study we have correlated every sample with proper history, predominant growth , sputum quality ,gram staining so this can solve the dispute.

Clinical features: [10]

Clinical features include increase in cough, chest pain, Increase in breathlessness, Increase in sputum volume and change in it's colour(white, green, yellow or blood streaked), Fever ,Increased fatigue ,Increase in oxygen requirement.

Physical findings-tachycardia, tachypnoea, expirtory wheeze, medium to coarse crackles, pursed lip breathing, central and peripheral cyanosis, neck vein distention, hepatomegaly, peripheral edema, hyperinflation of lungs with increased AP diameter of thorax and depressed diaphragm.

COMPLICATION:

- Hypoxemic Type 1 respiratory failure
- Hypercapnic Type 3 respiratory failure
- Compensated Metabolic alkalosis
- Cor pulmonale
- Left ventricular failure
- Multi organ failure
- Arterial oxygen desaturation
- Altered sensorium
- As per the National clinical guideline for the treatment of COPD [33].

LABORATORY DIAGNOSIS:

Proper diagnosis of AECOPD depends on clinical history, Physical examination and associated with Microbiological investigations. Microbiological investigations are important to isolate the organisms and to determine it's resistance pattern in order to prescribe the appropriate drug and to reduce the development new resistant strains and to prevent spread of existing resistant strains.

OTHER INVESTIGATIONS:

Pulmonary Function test, Complete blood count , Chest radiography, Complete metabolic profile, Arterial Blood gas analysis, Oxygen saturation, ECG, Echocardiography if required.

Microbiological investigations : [34,35,36,106,109]

The major benefit of microbiological investigations lies in the proper etiological diagnosis of AECOPD and to identify the resistance and susceptibility pattern of microorganisms. Different samples are collected from lower respiratory tract to perform different microbiological investigations. The most samples are:

A. Non invasive:

1. **Sputum (Expectorated):** Collected under direct supervision, to minimise contamination with oropharyngeal secretions.It is the easy and basic sample to assess the lower respiratory tract.

2. **Induced sputum:** Collected in patients who are unable to produce sputum. Those are the material directly obtained from alveolar spaces and should be accepted in laboratory without prescreening.

B. Invasive (Bronchoscopic techniques):

1. Bronchial washings
2. Bronchoalveolar lavage(BAL)
3. Protected catheter bronchial brush(rare sample)

Other Samples:

Endotracheal aspirate**:**

D. Blood :

As a routine investigations

A. Microscopy:

Direct microscopy examination aids to determine the quality of sample ,and to determine the severity and type of inflammatory response. To screen the likely pathogens directly in the clinical specimen and to correlate with the organisms grown in culture .With this initial screening ,the laboratory can help the clinicians to make early decisions to initiate antibiotic therapy early.

1. Staining methods :[34,35,36] Direct staining provides a differential staining and they enhance the chance of identifying the microbes and other details in the sample.

i) **Gram's stain**: it is meant for evaluating the quality of the specimen , presence of bacteria and it's arrangement, morphology, gram reactions , presence of neutrophils and also help to identify fungal elements .

ii) **Acid Fast stain**: Helps to identify acid fast bacilli directly in clinical samples

2. **10% Potassium hydroxide mount:** It is used to identify fungal elements in the specimen.

SCREENING OF SPECIMENS REQUESTED FOR ROUTINE BACTERIAL CULTURE TO ENSURE QUALITY [37,106,109]

SPECIMEN	SCREENING METHOD	ACCEPTABLE FOR CULTURE	NO FURTHER TESTING; request another sample
Sputum	Microscopic examination of gram stained smear	<10 squamous epithelial cells/average 10xfield	>10 squamous epithelial cells/average 10xfield
Endotracheal Aspirate	Microscopic examination of gram stained smear	<10 squamous epithelial cells/average 10xfield and bacteria seen in atleast 1 of 20 oil immersion fields	>10 squamous epithelial cells/average 10xfield and no bacteria seen in atleast 1 of 20 oil immersion fields
Bronchoalveolar lavage or Bronchial wash	Microscopic examination of gram stained smear	<1% of cells present are squamous epithelial cells	>1% of cells present are squamous epithelial cells

REQUIREMENTS FOR CULTURE AND CONDITIONS:[34,35,38]

Media	Incubation			Culture reading	Pathogens that grow
	Time	Temperature	Atmosphere		
5%sheep Blood agar	Upto 48 hrs	37 degree C	5-10% Co2	Daily	Streptococcus species, Staph.aureus, Moraxella Catarrhalis
Chocolate agar	Upto 48 hrs	37 degree C	5-10% Co2	Daily	Streptococcus spp.
5% Horse chocolate agar	Upto 48-72 hrs	37 degree C	5-10%CO2 with humidity	Daily	Haemophilus spp
MacConkey agar	Upto 48 hrs	37 degree C	Ambient air	Daily	Enterobacteriaceae, Non fermentors
Tryptic Soy broth	Upto 72 hrs	37 degree C	Ambient air	Daily	Blood culture broth
Sabouraud Dextrose Agar with antibiotics	Upto 4 weeks	25°C and 37°C	Ambient air	Daily for 1 week, twice weekly for next 3weeks weeks	Fungi

C) Antigen detection :

Counter current immunoelectrophoresis can be used to detect *Streptococcus pneumoniae* antigen in sputum. [56]

D) Serology: [40,41,42]

Indirect immunofluorescent assay for the simultaneous diagnosis in human serum of IgM antibodies of the main infectious agents of the respiratory tract is used for detection of atypical pathogens.Serologic assays for the detection of Specific Ig M antibody ,has been found to be very useful in detecting AECOPD caused by atypical pathogens and viruses ,which are difficult to grow by culture methods. It can be determined by ELISA. Biological markers like determination of Interleukines tumour necrosis factor α (TNFα), interleukin-1β (IL-1β), and the chemoattractants leukotriene B4 (LTB4), interleukin 8 (CXCL8), and growth-related oncogene α (GROα), Procalcitonin,C-reactive protein by ELISA .

E) Polymerase Chain Reaction :

PCR is an important technique which gives confirmatory result and higher diagnostic yield along with conventional diagnostic methods.PCR is useful for available for *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis,* and for many respiratory viruses

ANTIMICROBIAL THERAPY:[3,10]

Antibiotic prevention of exacerbations is a highly researched topic in COPD. Both the GOLD guidelines and European Respiratory society/European society of

Clinical Microbiology and Infectious disease(ESCMID) guidelines for lower respiratory tract infections management suggest that antibiotics should be given to AECOPD patients according to Anthosien criteria with1)all 3 cardinal symptoms.2)with either of two symptoms in which increased sputum purulence is one among. 3) mechanically ventilated patients .4)patients with severe COPD. The recommended duration for antibiotics is 3-7 days. Patients with type 1 and type 2 exacerbations are most likely to benefit from antibiotic therapy. Giving long term antibiotic treatment to a patient may have consequences; the development of antimicrobial resistance is by far the most important one. As the new emerging strains and the older strains develop emerging resistance pattern to old classes of antibiotics it has become still more important to determine antimicrobial sensitivity pattern.

Treatment should be worked towards three important goals particularly against bacterial infection:

1. Prevention of transient loss of pulmonary function
2. Relief of symptoms
3. To reassess the cause of disease to reduce the risk of further exacerbation

Drug treatment should always aim on decreasing the bacterial load, to prevent respiratory infection to decrease airway inflammation, to decrease work of breathing and to prevent further exacerbation ,to select proper and appropriate antibiotic therapy for flares of bacterial origin.

The Antibiotics should be chosen as per patients affordability, severity of exacerbation, bacterial spectrum, the most important is to have the knowledge of local bacteriological profile and it's sensitivity pattern especially prevalence of MRSA, ESBL, AmpC, MBL producers .

MDR are organisms which are resistant to 3 or more group of antibiotics with different mechanisms of action. Organisms isolated commonly with the following resistance pattern-[43]

1. Methicillin resistant *staphylococcus aureus* (MRSA)
2. Beta lactamases producing GNB-ESBLs, AmpC, MBL
3. Drug Resistance *Streptococcus pneumonia*e (DRSP).

1. BETA LACTAMASES IN GRAM NEGATIVE BACILLI:[44,45]

a) EXTENDED SPECTRUM BETA LACTAMASES (ESBL)

ESBL's are Bush class A plasmid mediated βlactamases capable of hydrolysing Penicillins and Monobactams and inhibited by βlactamase inhibitors(Clavulanic acid, Sulbactam, Tazobactam) but have no detectable activity against Cephamycins or Carbapenems(Imipenem, Meropenem) produced mainly by members of family enterobacteriaceae(*Klebsiella pneumonia,Klebsiella oxytoca,E.coli,Proteus mirabilis*) and some non fermentors.They also exhibit carry resistance for other group of antibiotics(like aminoglycosides, fluroquinolones, cotrimoxazole etc) which actually narrowes down the choices of antibiotics available for treatment.

Recent surveys have identified ESBLs in 70—90% of Enterobacteriaceae in India [105].Study done by SMART 2007 stated ESBL rates in India for *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were , 69.4%, and 100%, respectively which is higher as compared to our study.[46]

DETERMINATION METHODS FOR EXTENDED SPECTRUM BETALACTAMASES :[63]

1. Screening methods: using cefotxime/Ceftriaxone cefpodoxime /ceftazidime/ aztreonam discs by disc diffusion method.
2. CLSI phenotypic confirmatory methods: by broth microdilution method/disc diffusion method.
3. Other methods: Inhibitor potentiated disc diffusion test,double disc diffusion synergy test, ESBL E test, automated methods.
4. Molecular methods: PCR,DNA probes, PCR-RFLP,PCR-SSCP, Oligotyping, nucleotide sequencing.

b) AmpC PRODUCTION IN GRAM NEGATIVE BACILLI:[48]

Amp C βlactamases are Bush class C β lactamases (plasmid or chromosomal mediated),that are resistant to all beta lactamases (including Cephamycins) and are poorly inhibited by beta lactamase inhibitor (Clavulanic acid)combinations except Carbapenems.

The main Amp C producing microbes are *Acinetobacter species* and *Klebsiella* species.

Multicentric study conducted in India by Laghawe *et al* showed that the prevalenc of Amp C producing GNB in india is 15.97% .[47]

DETECTION METHODS FOR AmpC BETA LACTAMASES : [48]

1. Screening methods: using cefoxitin disc by disc diffusion method, Cefoxitin agar method, Inhibitor based methods, AmpC disc test, Modified three dimensional test, Amp C β lactamase E test.
2. Molecular methods: PCR based methods.

c) METALLO BETA LACTAMASES IN GRAM NEGATIVE BACILLI :[49]

MBL are Bush class B β lactamases capable of hydrolysing carbapenems, other β lactams and βlactamase inhibitors with the exception of aztreonam. They are predominantly found in *Acinetobacter baumanii* and *Pseudomonas aeruginosa.* Resistance to Carbapenems may be due to impermeability through cell wall due to loss of omrD porin, up regulation of an active efflux system ,Carbapenemase production ,or production of MBL's. In *Acinetobacter species* and *Pseudomonas aeruginosa* the prevalence of carbapenam resistance in various parts of our country was found to be 48-80% and 31 to 64% respectively.[50]

DETECTION METHODS FOR MBL :[64]

1. Screening methods:using carbapenem disc(imipenam, meropenam, ertapenam etc)
2. Confirmatory methods: Imipenam –EDTA combined disc method, Imipenam EDTA double disc synergy test (DDST),EDTA disc potentiation test, HODGE test, MBL E test
3. Molecular methods: PCR techniques

2. METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*:

These are β lactamases which rendeer the organism resistance to all available βlactamases, βlactam/βlactamase inhibitor combinations, monobactams abd carbapenems. A study conducted by INSAR group ,showed that the prevalence of MRSA in our country is about 40 %.[51]

Detection methods for MRSA [52]:

1. Screening methods: using cefoxitin/oxacillin disc by disc diffusion method
2. Confirmatory methods:Oxacillin MIC detection (by broth dilution,agar dilution,E test method),Oxacillin screen agar
3. Molecular methods:detection of mecA gene or PBP2protein(its protein product)

3. DRUG RESISTANT *STREPTOCOCCUS PNEUMONIAE* [53]

In the past, S. pneumoniae was almost uniformly susceptible to penicillin, allowing most physicians to treat persons who had severe infections with penicillin alone without testing for resistance. Resistance to penicillin and other antimicrobial agents has spread rapidly and was first reported in Australia in 1967, in New Guinea in 1969, in South Africa in 1977, and in many other countries throughout Africa, Asia, and Europe.MDR strains resistant to penicillin, tetracycline, erythromycin, cotrimoxazole and chloramphenicol were identified.

Investigations of outbreaks by CDC have revealed that Pneumococcal isolates resistance to penicillin in some areas of the United States, as many as 30%.[54]

In a study conducted by Kurien et al. Pneumococcal infection is found in the prevalence of about 53.90% in India, with resisitance strains ranging from 4%-15.12%,in different parts of our country .[55]

DETECTION METHODS FOR DRUG RESISTANT *TREPTOCOCCUS PNEUMONIAE* : [56]

1. Screening method : by disc diffusion method using MHA supplemented by 5% sheep blood.Penicillin sensitivity is detected by using oxacillin (1µg) disc.
2. Confirmatory methods: MIC detection methods – Isolates found to be nonsusceptible by oxacillin disk should then be subjected to quantitative MIC testing against penicillin. MIC detection methods –broth/agar dilution method, or antimicrobial gradient E strips using Mueller Hinton broth supplemented with lysed horse blood or defibrinated sheep blood.

AIMS AND OBJECTIVES OF THE STUDY

AIMS AND OBJECTIVE

- To identify the bacterial and fungal agents causing acute exacerbation in COPD and in Stable COPD .
- To compare the isolates between AECOPD and Stable COPD patients
- To determine the antibacterial and antifungal susceptibility pattern of the isolates in both the cases.

MATERIALS AND METHODS

MATERIALS & METHODS

This study was conducted at the Institute of Microbiology, Madras Medical College in association with Departments of InternalMedicine, Thoracic Medicine, Intensive Medicare Care Unit at Rajiv Gandhi Government General Hospital, Chennai. This study samples were selected as per GOLD criteria[14] .

Study design & period:

Cross sectional study. One year (from October 2013 to September 2014)

Study population:

The Study population consisted of 150 in patients presenting with signs and symptom of AECOPD and 50 stable COPD out patients.

Ethical clearence:

Before the commencement of the study, approval was obtained from Institutional Ethical Committee. Informed consent was obtained from the study group. The Patients were interviewed with structured questionnaire.

Inclusion criteria:

Patients older than 18 years were selected as per FEV1/FVC and FEV1 with GOLD criteria[14] Among these the following patients were selected;

For AECOPD as per Anthosien criteria [15]

- Patients with history of chronic bronchitis , emphysema, and small airway disease presenting with two of the following symptoms of acute exacerbation such as:
- Increased cough
- Increased purulence and/or volume of expectorations
- Increased severity of dyspnoea.
- 4.Fever and Leucocytosis

For Stable COPD patients

- Patients with history of COPD visiting OPD without the above symptoms of AECOPD were included in the Stable group.

Exclusion criteria:

- Patients with asthma, interstitial lung disease, present or past history of tuberculosis and all cases who had evidence of pneumonia or bronchiectasis clinically or on chest radiography (PA view) which developed as a sequelae to other disease.
- Exacerbation due to noninfectious causes (pulmonary thromboembolism, air pollutants).
- Patients already taking antibiotics for past 24 hrs .

Collection of data:

Data were collected from patients who satisfied the inclusion criteria, using preformed structured questionnaire. Demographic details like name, age sex,

address,date of admission, clinical data like presenting complaints, personal history, past medical history, history suggestive ofimmunization , chest radiographic findings, physical examination findings and details of clinical diagnosis were collected.

Basic blood investigation such as Haemoglobin estimation, Total leukocyte count, Differential count,ESR,Blood glucose level,Blood urea and Serum creatinine levels were documented.

Sample collection and Transport:

All the samples were collected under strict aseptic precautions in sterile containers, properly labelled and were transported to the laboratory in appropriate conditions and processed within one hour of collection.

Samples collected:

1) Sputum (expectorated /induced)
2) Bronchial wash
3) Endotracheal aspirate
4) Blood

Procedure of collection &transportation of samples[21,106,109]

1a) Expectorated Sputum:

The patient was instructed regarding the method of using the sterile sputum cups and the importance of collecting deeply coughed specimen. Patients were instructed to brush their teeth , rinse their mouth with saline or water, just before collecting sputum sample. Early morning sputum samples were collected under direct

supervision even before the patient had any food intake. Samples were taken to the laboratory for processing, within 1 hour of collection.

1b) Induced sputum:

These samples (aerosol induced specimen) were collected after allowing the patients to inhale aerosolized droplets of solution containing 15% sodium chloride for 10 minutes or until a strong cough reflex is induced. The samples were collected in sterile sputum cups and were taken to the laboratory for processing, as soon as possible.

2) Bronchial wash:

These samples were collected when the pulmonologist, via a fiber optic bronchoscope, was not able to visualize purulent secretions or diseased part of the lung segment. Small amounts of physiological saline were infused and the reaspirated sample is collected into the sterile container for further processing.

3) Endotracheal aspirate:

The fraction of inspired oxygen was set at 90% or more. None of the patients received local anesthetics. A blind endotracheal aspiration sample was obtained first by sterile means using a 22-inch suction catheter and collected in a mucus collector (Specimen trap) for patients with endotracheal tube.

4) Blood:

With strict aseptic precautions 10ml of blood sample was collected into a sterile screw capped blood culture bottle containing 50 ml of sterile Trypticase Soy Broth.

Direct Microscopy:

All the respiratory samples like sputum, bronchial wash,Endotracheal aspirate were subjected to the following microscopic examination as per Standard Operating Procedures.

- **10% potassium hydroxide mount**-to detect the presence of fungal elements
- **Gram's stain**-to detect the presence of bacterial cells, their gram reaction, morphology, arrangement and also to detect fungal elements
- **Acid Fast stain**-to detect the presence of Mycobacterium tuberculosis bacilli in the Sample

Processing of sample and Culture : [35-37]

1) Sputum: [55,39,56]

A few glass beads (2.5-3.5 mm) and an equal volume of 2% (w/v) N-acetyl-l-cysteine (NAC) were added to each specimen. The NAC solution was freshly prepared each day by dissolving 2 g NAC in 13 ml 1N NaOH and diluting to a final volume of 100 ml with PBS. The pH of the solution was adjusted to 7.3 . The caps on the universal containers were securely tightened, the NAC-sputum mixtures were agitated on a vortex mixer for 10 seconds, allowed to stand at room temperature for 10

minutes, and finally vortex mixed for a further 15 seconds. Homogenised sputa were processed within 30 minutes.

All the sputum samples were prescreened with Gram's stain , using Bartlett scoring system. Only those samples which met the acceptance criteria (a final score 0f >0) were further processed for culture. Rest of the samples was discarded and repeat sample was obtained in all possible cases.

Sputum samples were mechanically homogenized with sterile glass beads using vortex machine. Tenfold serial dilutions of the homogenised sample were made in brain heart infusion broth and with 0.01 ml loop were plated out onto the surface of a range of different media including blood agar, chocolate agar, MacConkey agar.

- MacConkey agar plate, at 37 oC in ambient air for 24 hrs
- 5% sheep Blood agar plate, with 5-10% Co2, 37 o C for 24 hrs
- Chocholate agar plate ,with 5-10% Co2, 37 o C for 24 hrs
- 5% Horse chocolate agar plate, with 5-10% CO2, at 37^0 C for 24 hrs

Horse Blood Collection:

Horse was brought to plasmapheresis block and name,group,bleeding date were verified before bleeding. Jugular vein was raised by means of a neck rope .The area at the site of jugular furrow was shaved and sterilised .A cannula of 10G autoclaved needle is inserted into the vein .The blood was collected by using sterilised 5L/10L glass bottle with 30-40 glass beads.

(As per CPCSEA norm – Blood collected as per body weight of animal :0-5% of body wt every 2 wks with plasmapheresis or 1.5% of body wt for every 4 wks with plasmapheresis ie 300ml)

After incubation, bacterial colonies were counted .The number of colony forming units/ml sputum was calculated from the number of colonies obtained and the dilution of the sputum.

Evaluation of bacterial culture plates:

Colonies grown on cultured plates were evaluated at 24 and 48 hrs

a) Detection of Colony Forming Units in the sample:[38]

After the specified period and condition of incubation, the number of colonies grown in the culture plate is counted and the CFU/ml in the original sample was calculated using the following formula,

CFU/ml=number of colonies X dilution factor

CFU $>10^6$/ml was accepted as potential pathogen except for *Streptococcus pneumoniae* CFU $>10^5$/ml was considered as significant.

Induced sputum sample was accepted for culture without prescreening , as they usually contain material directly from alveolar spaces with very little contamination from upper respiratory secretions .

2) Bronchial wash:

These samples were concentrated by centrifugation and the sediment were used for Gram staining and culture. Prescreening is not done for these samples,as they contain lower respiratory tract material.

3) Endotracheal Aspirate(EA)[57]:

Endotracheal aspirate samples were mechanically liquefied and homogenized by vortexing for 1 min with glass beads, followed by centrifuging at 3,000 rpm for 10 min .EA cultures were quantified using calibrated loops. 100-fold diluted EA were evenly streaked with 1 µl loop on entire surface of a chocolate agar plate, a sheep blood agar plate, and a MacConkey agar plate. Plates are incubated overnight in a 5% CO_2 atmosphere at 35°C. Colonies were then counted and bacterial concentrations (cfu/mL) were calculated. Microorganisms with counts > 10^4 cfu/mL were submitted for identification and antimicrobial susceptibility testing. If no growth was detected on any plate, the incubation was extended for 24 hr.

4) Blood:

Blood sample along with Trypticase Soy Broth in blood culture bottle is incubated for 24 hrs at 37oC and then further subcultured onto specific media plates.

- For fungal culture the samples were inoculated into two sets of culture media- Sabouraud's Dextrose agar (SDA) with cycloheximide and antibiotics, incubated at 25°C and at 37°C,for upto 4 weeks.

b) Interpretation of bacterial cultures:

The isolated colonies were identified by means of Gram's stain, motility, catalase test, oxidase test, coagulase test and by of various other biochemical reactions like Indole test, Methyl red test, Voges proskauer test, Citrate utilisation test, Urease test, Triple sugar iron agar, Nitrate reduction test, Hugh-Leifsons oxidation fermentation test, coagulase production (for *Staphylococcus*), Optochin Sensitivity (for *Streptococcus pneumoniae*)were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and Dulcitol, inositols etc were done to identify the isolate according to standard laboratory procedures.

c) Interpretation of fungal cultures:

Inoculated SDA slants were inspected daily for first one week and then twice weekly for the next 3 weeks. Filamentous fungal isolates were identified by LPCB mount preparation, based on the hyphal and conidial arrangement and morphology.

Antimicrobial susceptibility testing : [58]

Done to identify the sensitivity and resistant patterns of all isolates according to CLSI guidelines.

Non fastidious organisms[59,60]:

Antimicrobial susceptibility testing was done by disc diffusion method using Kirby bauer technique on Mueller Hinton agar (HiMedia,Mumbai) ,using appropriate antimicrobial drugs as directed by CLSI guidelines.

- Inoculum: Growth method suspension, equivalent to a 0.5 McFarland standard Incubation: 35 ± 2 °C; ambient air ,for 16 to 18 hours
- Quality control tests were done every week for testing the performance of media & drugs using the following standard ATCC control strains.

ATCC control strains:

- *Staphylococcus aureus*–ATCC 25923
- *Escherichia coli*-ATCC 25922
- *Pseudomonas aeruginosa*-ATCC 27853
- *Klebsiella pneumoniae* (ESBL)-ATCC 700603

Interpretation of Zone of inhibition diameters were done according to CLSI guidelines.

Fastidious organism[61,62]:

Antimicrobial susceptibility testing was done by disc diffusion method using Kirby bauer technique on Mueller Hinton agar supplemented with 5% sheep blood , using antimicrobial drugs ,as directed by CLSI guidelines .

- Inoculum: Direct colony suspension, equivalent to a 0.5 McFarland standard
- Incubation: 35 ± 2 °C; in 5% CO2 for 20 to 24 hours

Quality control tests were done every week for testing the performance of media & drugs using the standard *Streptococcus pneumonia* ATCC49619 control strain.

Interpretation of Zone of inhibition diameters were done according to CLSI guidelines for all isolates .

Panel of antibiotics included for testing antimicrobial sensitivity of Gram negative bacilli.

Antibiotic	Disc content in µg	Gram Negative Bacilli	Diameter of Zone of Inhibition in mm Break points		
			Sensitive	Intermediate	Resistant
Amikacin	30		≥17	15-16	≤14
Cefotaxime	30	*Enterobacteriaceae*	≥26	23-25	≤22
		Acinetobacter	≥23	15-22	≤14
Ceftazidime	30	*Enterobacteriaceae*	≥21	18-20	≤17
		Acinetobacter *P.aeruginosa*	≥18	15-17	≤14
Cotrimoxazole	1.25/23.75		≥16	11-15	≤10
Ciprofloxacin	5		≥31	21-30	≤20
Gentamicin	10		≥15	13-14	≤12
Imipenem	10	*Enterobacteriaceae*	≥23	20-22	≤19
		P.aeruginosa	≥19	16-18	≤15
		Acinetobacter	≥22	19-21	≤18
Piperacillin-Tazobactam	100/10	*Enterobacteriaceae* *Acinetobacter*	≥21	18-20	≤17
		P.aeruginosa	≥21	15-20	≤14

Panel of antibiotics included for testing antimicrobial sensitivity of Gram positive and Gram Negative Cocci.

Antibiotic	Disc content µg	Organisms	Diameter of Zone of Inhibition in mm Break points		
			Sensitive	Intermediate	Resistant
Amikacin	30		≥17	15-16	≤14
Penicillin	10units	*Staphylococcus aureus*	≥29	-	≤28
Amoxycillinclavulanic acid	20/10	*Moraxella catarrhalis*	≥20	-	≤19
Ciprofloxacin	5		≥21	16-20	≤15
Cotrimoxazole	1.25 / 23.75	*Staphylococcus aureus*	≥16	11-15	≤10
		S.pneumoniae	≥19	16-18	≤15
Cefotaxime	30	*Streptococcus sp.*	≥24	-	-
Chloramphenicol	30	*S.pneumoniae*	≥21	-	≤20
		Streptococcus sp	≥21	18-20	≤17
Cefoxitin	30	*Staphylococcus aureus*	≥22	-	≤21
Erythromycin	15	*Staphylococcus aureus*	≥23	14-22	≤13
		S.pneumoniae	≥21	16-20	≤15
Ofloxacin	5	*S.pneumoniae*	≥16	13-15	≤12
Oxacillin	1	*S.pneumoniae*	≥20	-	-
Optochin	5	*S.pneumoniae*	≥14	-	<14
Tetracycline	30	*Staphylococcus aureus*	≥19	15-18	≤14
		S.pneumoniae	≥28	25-27	≤24
Vancomycin	30	*S.pneumoniae*	≥17	-	-

Methods of detection of β lactamase production among gram negative bacilli:

A)Extended Spectrum β Lactamase - detection methods : [44,45]

1)Screening test:

Gram negative bacilli isolates showing the following zone of inhibition diameters to the respective drugs were considered to be possible ESBL producers.

Antibiotic Break point zone diameter for possible ESBL strains

Cefotaxime(30μg) ≤27 mm

Ceftazidime(30μg) ≤22 mm

2) Phenotypic confirmatory method:

To 5ml of nutrient broth , 3-5 colonies of isolates grown on a non selective culture medium was added and incubated for 2-4 hrs at 35o C and the resulting turbidity is matched with 0.5 Mcfarlands standard. The test was lawn cultured onto Cation adjusted MHA Plate(HiMedia,Mumbai). Ceftazidime (30μg) disc and Ceftazidime/Clavulanic acid disc (30μg/10μg) (Himedia, Mumbai) were placed on the surface of the plate and incubated overnight at 35^0 C. A ≥ 5mm increase in zone diameter for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone confirmed an ESBL producing organism.

3) Double disk diffusion synergy test:

On a lawn culture of 0.5 Mcfarlands test lsolate on Cation adjusted MHA plate, Augmentin disc(Amoxycillin Clavulanic Acid) was placed in the centre of the plate and a disc of Ceftazidime (Himedia, Mumbai) was kept 30mm apart from centre to

centre .After incubation , a clear increase in the zone of inhibition towards Augmentin disc was interpreted as positive for ESBL production.

B)AmpC β lactamases detection methods : [48]

1) Screening method:

A lawn culture of 0.5 Mcfarland suspension of test isolate was made on Cation adjusted MHA plate. Ceftazidime (30µg) disc were placed adjacent to Cefoxitin (30µg) disc at a distance of 20 mm from each other. After overnight incubation at 35^0 C, isolates showing blunting of Ceftazidime zone of inhibition adjacent to Cefoxitin disc or showing reduced susceptibility to Ceftazidime and Cefoxitin were considered as screen positive.

2) AmpC disc test :

On a Cation adjusted MHA plate ,lawn culture of ATCC E.coli 25922 was prepared.On a 6mm sterile disc ,which are moistened with sterile saline, several colonies of test organism were inoculated. The inoculated disc was then placed beside a Cefoxitin disc (30µg) (almost touching) on the inoculated plate. After incubation, flattening or indentation of the Cefoxitin inhibition zone in the vicinity of the test disc were considered as AmpC positive isolate.

C) Metallo β lactamase (MBL) detection methods : [49]

1) Imipenem- EDTA double disc synergy test(DDST):

The organism was inoculated onto Cation adjusted MHA plates as recommended by the CLSI .A 10 µg Imipenem disc was placed 20mm centre to centre from a blank disc containing 10µl of EDTA .After incubation there was

enhancement of zone of inhibition in the area between Imipenem and EDTA as compared to the zone of inhibition on the far side of drug was interpreted as positive.

2) Imipenem –EDTA combined disc test:

Imipenem 10µg and 10µg Imipenem disc containing 750µg of EDTA solution, were placed on a lawn culture of test organism on Cation adjusted Muller Hinton Agar plate and incubated overnight. If the increase in inhibition zone with Imipenem-EDTA disc was ≥ 7mm than the Imipenem disc alone, it was considered MBL positive.

1) Screening and confirmatory test for suspected carbapenase production: [64]

A) Initial screen test :

0.5 Mcfarland's suspension of test isolate was lawn cultured on cation adjusted MHA plates. 10 µg Meropenem disc was placed on the surface of lawn culture, incubated at 33–35 °C; in ambient air for 16–18 hours.

Interpretation : Meropenem 16-21mm

The zone diameter of inhibition indicates Carbapenamase production and was confirmed by Modified Hodge test.

B) **Phenotypic confirmatory test: Modified Hodge test(MHT)**

It is a phenotypic confirmatory test for Carbapenemase production in Enterobacteriaceae.

The Confirmatory test recommendations are largely derived from US isolates of *Enterobacteriaceae* ,and provide a high level of Sensitivity(>90%) and specificity

(>90%) in detecting KPC-type carbapenemase in these isolates .No data exist on the usefulness of these tests for the detection of Carbapenemase production in non-fermenting gram –negative bacilli.

Method:

0.5 Mcfarland's suspension of ATCC E.coli 25922 isolate in broth or saline ,and dilute 1:10 in saline or broth was lawn cultured on cation adjusted MHA plates. 10 µg Meropenem disc was placed on the surface of lawn culture .With 10 µl loop 3-5 colonies of positive control *Klebsiella pneumoniae ATCC –BAA-1705* ,of negative control *Klebsiella pneumoniae ATCC –BAA-1706* and of test isolates grown overnight on Blood agar plate were inoculated in a straight line out from the edge of the disc .The streak length of 20-25mm were made. Incubated at 33–35 °C; in ambient air for 16–18 hours.

Interpretation : The presence of distorted zone of inhibition was interpretated as positive for Carbapenemase production.

Note :Not all Carbapenemase producing isolates of Enterobacteriaceae are MHT positive and MHT –positive results may be encountered in isolates with Carbapenem resistance mechanisms other than Carbapenemase production.

Methicillin resistance detection in *Staphylococcus aureus* : [65]

Disc diffusion method:

0.5 Mcfarland's suspension of test isolate was lawn cultured on MHA plates.30 µg cefoxitin disc is placed on the surface of lawn culture ,incubated at 33–35 °C; in ambient air for 16–18 hours.Isolates showing inhibition zone diameter ≥22 mm ,were considered as Methicillin sensitive strains and those that show inhibition zone diameter ≤21 mm ,were considered as Methicillin resistant isolates.

Detection of Vancomycin MIC for *Staphylococcus aureus* isolates by macrobroth dilution methods :[104]

Cation adjusted Mueller Hinton broth.(pH 7.2-7.4)

Media Preparation of stock antibiotic solution:

Formula:

$$W = \frac{1000 \times V \times C}{P}$$

where

W = weight of the antibiotic to be dissolved in the volume V

V = volume of the stock solution to be prepared (10ml)

C =final concentration of the antibiotic solution (1024µg/ml)

p= potency of the antibiotic in relation to the base. (For vancomycin, p= 950/1000 mg; Himedia)

10.8mg of meropenam drug was dissolved in 10ml of distilled water to prepare a stock solution concentration of 1024µg

Preparing dilution of antibiotics:

- Arrange two rows of sterile test tubes (1 row for the test & 2nd for ATCC
- control from conc 1024-0.0625 μg/ml)
- Using sterile pipette put 1ml cation adjusted MH broth in each 12 test tubes of both ATCC control and test .
- Take 1ml from stock solution and put it in 1 st test tube of both control and test
- Then take 1ml from 1st test tube to the 2nd test tube and repeat this procedure of two fold serial dilution till the 12th tube .
- Place 1 ml of the antibiotic free broth in the last tube in each row (growth control)
- The sterility controls for the antibiotic solution was also kept.

Inoculum preparation for the test and ATCC control and incubation:

- To 9.9 ml of MH broth in a test tube , add 0.1 ml of 0.5 Mcfarland turbidity matched test organism.
- Mix well, transfer 1 ml of inoculum using 2 ml pipette to each tube containing antibiotic dilutions and also to the control tube.
- Similarly repeat the procedure for ATCC control strain
- Incubate the rack at 37 ^{0}C for 24hrs

Observation &Interpretation:

- Observe the MIC of ATCC control strain, If it is out of the sensitive range, then test is invalid.
- If MIC of ATCC strain is in the sensitive range then read for the test organism
- The lowest concentration of the antibiotic in which there is no visible growth will be the MIC for the drug & for the test organism.

Interpretation criteria: MIC values for Vancomycin was interpreted as follows:

MIC ≤ 2μ/ml - Sensitive.

MIC : 4-8 μg/ml - Intermediate

MIC : ≥16μg/ml - Resistant

Detection of MIC by microbroth dilution method, for Meropenem ,in case of drug resistant *Enterobacteriaceae and Non Enterobacteriaceae* -isolates [104]:

Culture media: Cation adjusted Mueller Hinton broth (pH 7.2-7.4)

Preparation of stock antibiotic solution:

Formula:

$$W = \frac{1000 \times V \times C}{P}$$

where

W = weight of the antibiotic to be dissolved in the volume V

V = volume of the stock solution to be prepared (10ml)

C =final concentration of the antibiotic solution

p= potency of the antibiotic in relation to the base

Potency of Meropenam=675/1000mg

15.2mg of meropenam drug was dissolved in 10ml of distilled water to prepare a stock solution concentration of 1024µg/ml

Preparing dilution of antibiotics:

- Arrange two rows of sterile test tubes (1 row for the test & 2nd for ATCC
- control from conc 1024-0.0625 µg/ml)
- Using sterile pipette put 1ml cation adjusted MH broth in each 12 test tubes of both ATCC control and test .
- Take 1ml from stock solution and put it in 1 st test tube of both control and test .
- Then take 1ml from 1st test tube to the 2nd test tube and repeat this procedure of two fold serial dilution till the 12th tube .
- Place 1 ml of the antibiotic free broth in the last tube in each row (growth control)
- The sterility controls for the antibiotic solution was also kept.

Inoculum preparation for the test and ATCC control and incubation:

- To 9.9 ml of MH broth in a test tube , add 0.1 ml of 0.5 Mcfarland turbidity matched test organism.
- Mix well, transfer 1 ml of inoculum using 2 ml pipette to each tube containing antibiotic dilutions and also to the control tube.
- Similarly repeat the procedure for ATCC control strain
- Incubate the rack at 37 ^{0}C for 16-20hrs

Observation &Interpretation:

- Observe the MIC of ATCC control strain, If it is out of the sensitive range, then test is invalid.
- If MIC of ATCC strain is in the sensitive range then read for the test organism
- The lowest concentration of the antibiotic in which there is no visible growth will be the MIC for the drug & for the test organism.

Interpretation criteria: for **Meropenam MIC values** *Enterobacteriaceae*

- MIC ≤1μg/ml –sensitive.
- MIC :2 μg/ml –Intermediate
- MIC :≥4μg/ml-resistant

Meropenam MIC values for *Non- Enterobacteriaceae*

- MIC: ≤2μg/ml –sensitive.
- MIC :4μg/ml –Intermediate
- MIC :≥8μg/ml-resistant

Drug resistant *Streptococcus pneumoniae* : (98.103)

1. Screening method : by disc diffusion method using MHA supplemented by 5% sheep blood. Penicillin sensitivity is detected by using oxacillin (1μg) disc

2. Confirmatory methods: Isolates found to be nonsusceptible by oxacillin disk should then be subjected to quantitative MIC testing against penicillin. MIC

detection methods –broth/agar dilution method, or antimicrobial gradient E strips using Mueller Hinton broth supplemented with lysed horse blood or defibrinated sheep blood.

Note : a) For fastidious organisms,if the diameter of the zone of inhibition falls within the resistant range,ie ≤19mm it is confirmed by MIC detection for the concerned drugs before reporting it as resistant, as per CLSI guidelines.

b) Penicillin susceptibility were detected by using Oxacillin(1μg) disc. A zone diameter of ≥20mm for oxacillin is taken as penicillin susceptible strains .

Method:

Detection of Penicillin Minimum inhibitory concentration for *Streptococcus pneumoniae* isolates by Epsilometer test (E test) in case of resistance to Oxacillin disc: [98]

Streptococcus pneumoniae was grown overnight on blood agar plates, suspended in Trypticase soy broth to a 0.5 MacFarland density, and inoculated for confluent growth onto Mueller-Hinton-blood agar plates. Penicillin E test strips were applied to the plates after the inoculum had dried for 15 min. The penicillin E test strips used had a MIC range of 0.002 to 32 ,ug/ml. Incubation was at 35°C, under 5% CO2 for 18 to 24 h. The MICs were read at the point of intersection between the edge of the zone of bacterial growth and the E test strip, per manufacturer's instructions.

MIC Observation and interpretation as follows:

MIC ≤0.06 µg/ml- Sensitive

0.12-1 µg/ml-Intermediate

≥2µg/ml- Resistant

STATISTICAL ANALYSIS:

Statistical analysis was done by using statistical package for social sciences(SPSS) version 21.The test used in this study was Pearson's Chi square analysis test.P value less than <0.05 is considered as stastically significant.

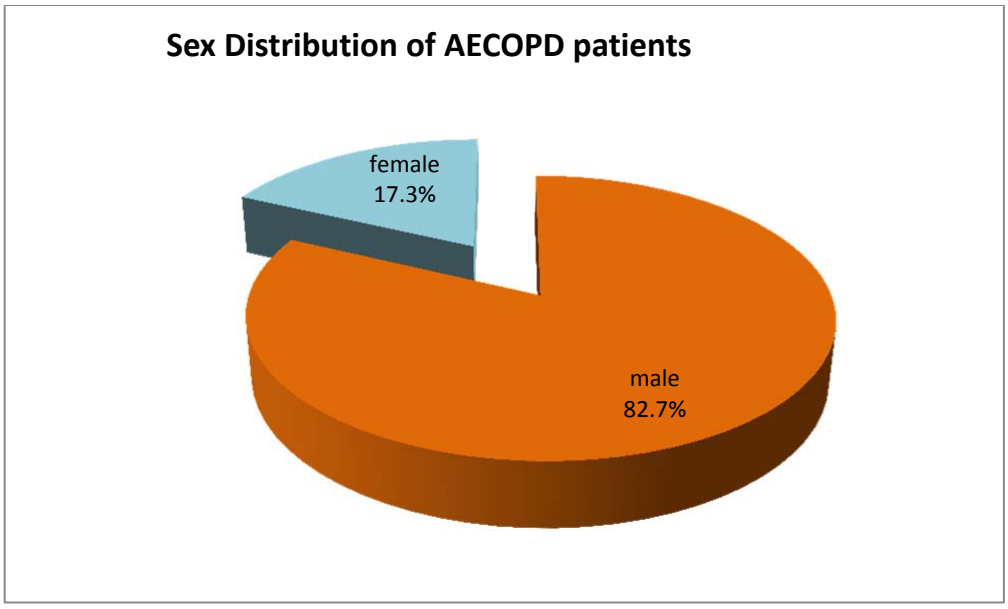
RESULTS

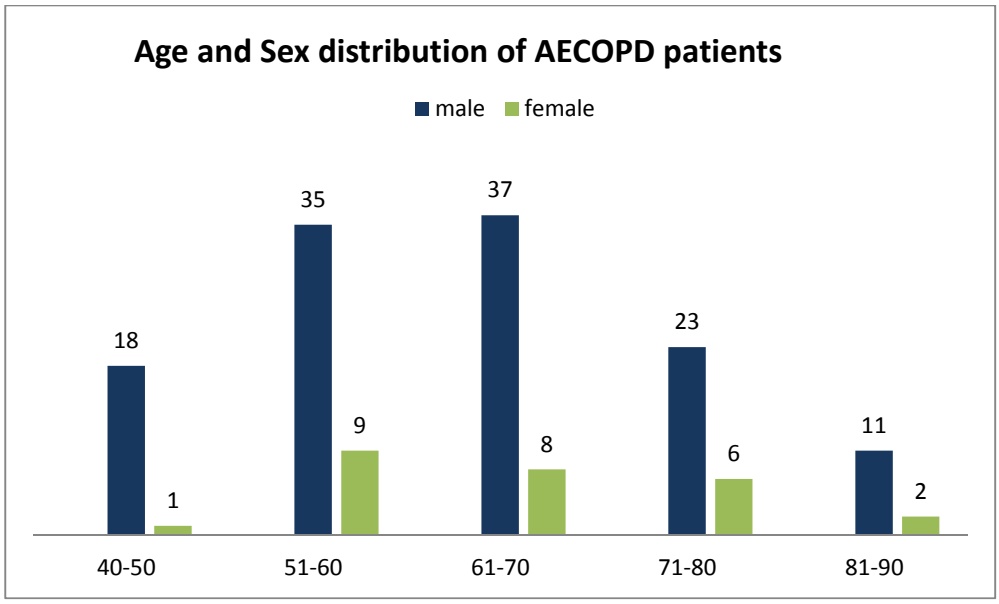
RESULTS

This Cross sectional study was conducted at the Institute of Microbiology, Madras Medical College in association with Departments of Thoracic Medicine,Internal Medicine and Intensive Care Unit at the Rajiv Gandhi Government General Hospital, Chennai. A total of 200 patients were included in the study who were categorized into two groups-150 AECOPD and 50 Stable COPD patients. None of the patients had received immunization for *Haemophilus influenzae* or *Streptococcus pneumoniae* in the past.

TABLE 1 : AGE AND SEX DISTRIBUTION OF AECOPD (n=150)

AGE IN YEARS	MALE	FEMALE	TOTAL	PERCENTAGE
40-50	18	1	19	12.7%
51-60	35	9	44	29.4%
61-70	37	8	45	30%
71-80	23	6	29	19.3%
81-90	11	2	13	8.6%
TOTAL	124(82.7%)	26(17.3%)	150	100%

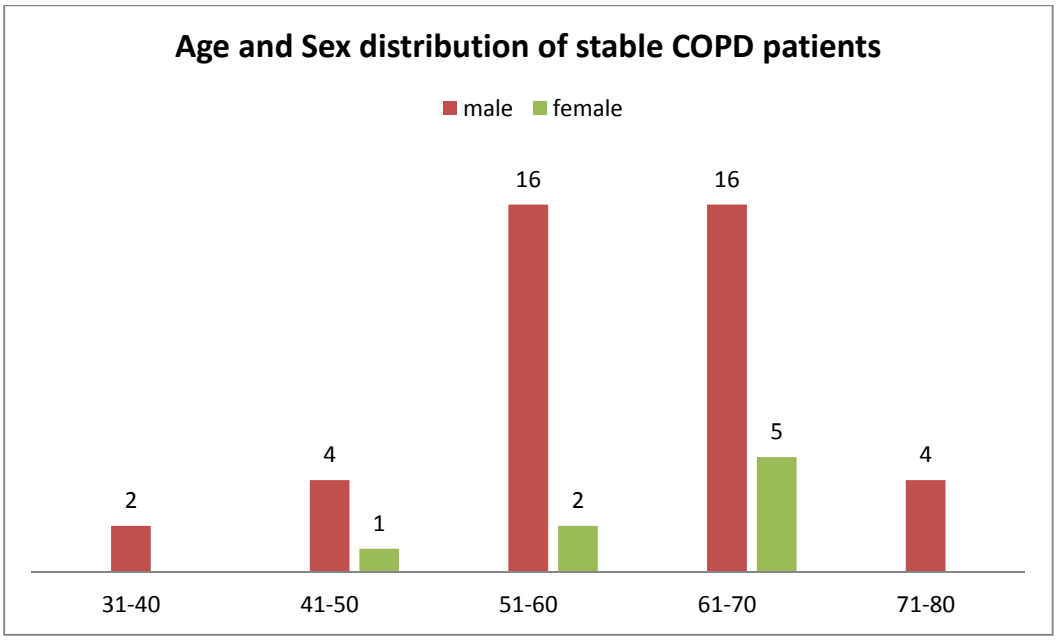


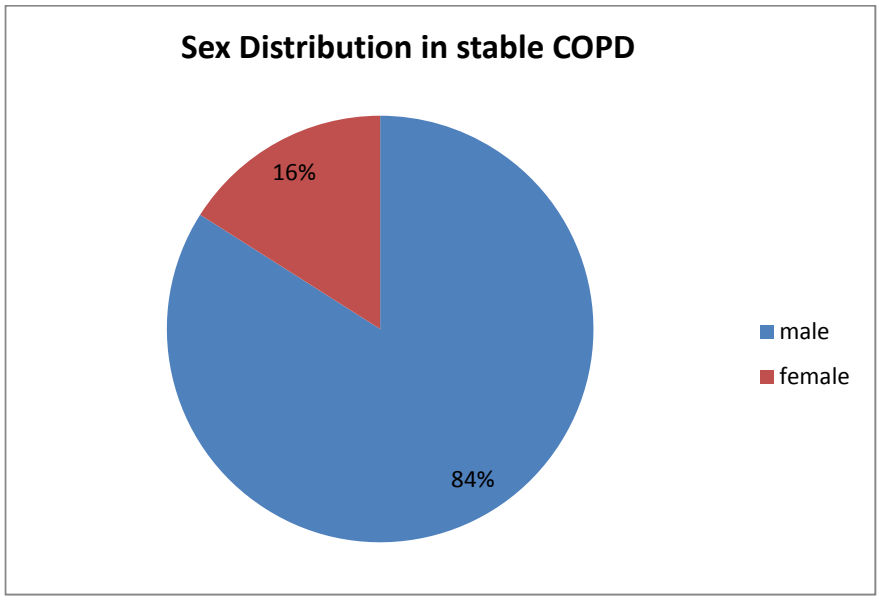


In AECOPD the study group consisted of predominantly males with a male:female ratio 62:23.

TABLE 2 : AGE AND SEX DISTRIBUTION OF STABLE COPD PATIENTS (n=50)

AGE IN YEARS	MALE	FEMALE	TOTAL	PERCENTAGE
31-40	2	0	2	4%
41-50	4	1	5	10%
51-60	16	2	18	36%
61-70	16	5	21	42%
71-80	4	-	4	8%
TOTAL	42(84%)	8(16%)	50	100%





In stable COPD the study group consisted of predominantly males with a ratio of 21:4

TABLE 3: ASSOCIATION OF SMOKING AS THE RISK FACTORS OF COPD AMONG STUDY GROUP

		AECOPD n=150		Stable COPD n=50	
		No	**%**	**no**	**%**
1	Smoking	122	81.3	42	84
2	Non -smoking	28	18.7	8	16

Smoking was associated with AECOPD in 81.3% of cases and 84% in COPD patients. There was statistical significance P value <0.001.

TABLE 4: ASSOCIATION OF OCCUPATION AS RISK FACTORS OF COPD AMONG STUDY GROUP

		AECOPD n=150		COPD n=50	
		No	**%**	**No**	**%**
1.	Biogas inhalation during cooking/burning fire	26	17.3	8	16
2.	Carpenter	1	0.6	-	-
3	Aluminium worker	1	0.6	1	2

Biogas inhalation was associated with AECOPD in 17.3% of cases and 16% among COPD cases. Occupation as a risk factor was found in 6% of cases in AECOPD and 2% in Stable COPD. There was no Statistical significance

ANALYSIS OF CAUSES OF COPD IN AECOPD PATIENTS

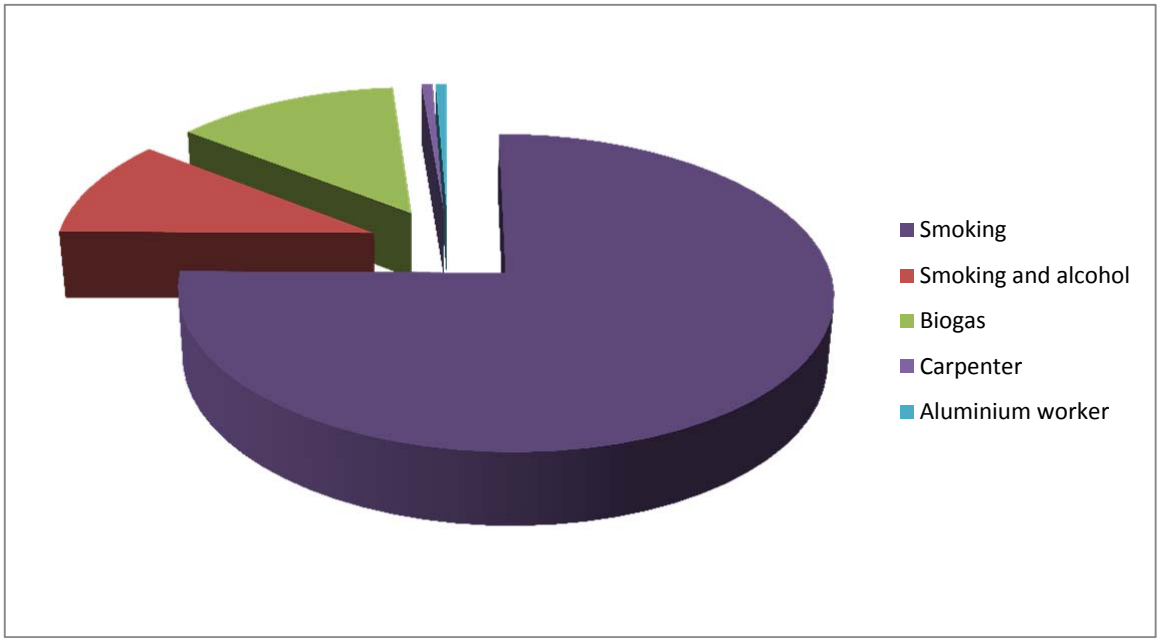


TABLE 5 : DISTRIBUTION OF STUDY POPULATION AS PER CLINICAL DIAGNOSIS

AECOPD n=150			STABLE COPD n=50	
TYPE	**No**	**%**	**No**	**%**
EMPHYSEMA	7	4.6	3	6
CHRONIC BRONCHITIS	143	95.3	47	94

Chronic bronchitis was the most common condition in both stable (95.3%) and acute exacerbation(94%) of COPD.

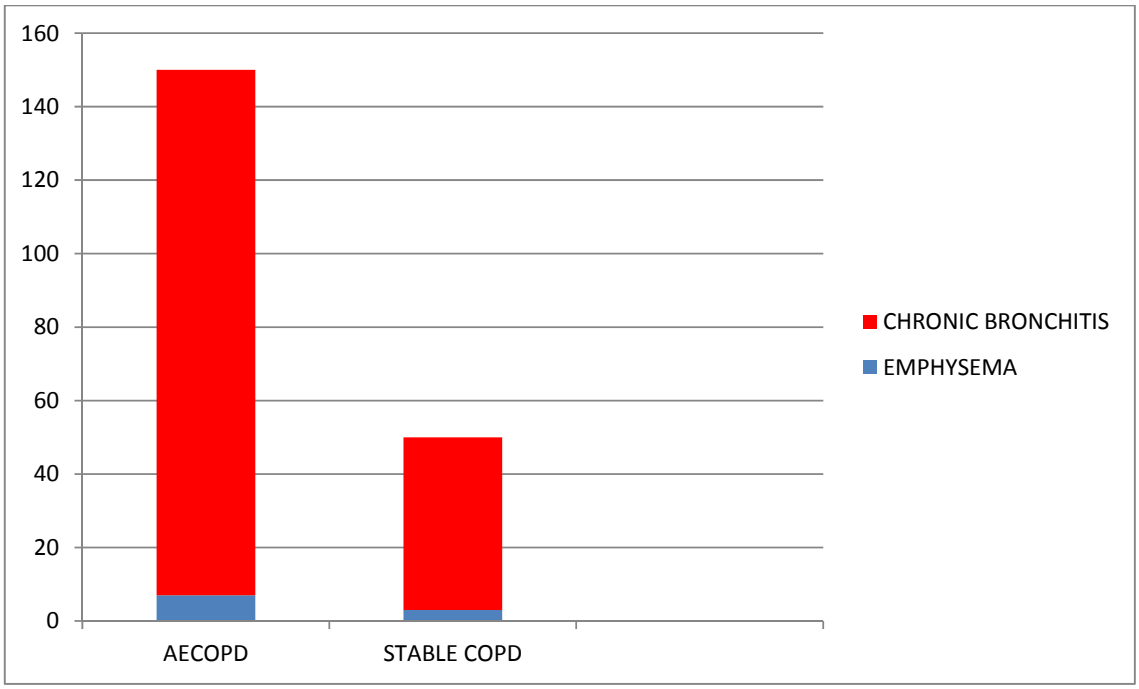


TABLE 6: ANALYSIS OF SYMPTOMS OF PATIENTS WITH AECOPD (n=150)

S. No	Symptoms	No	%
1	Increased Sputum volume	120	80%
2	Increased Sputum purulence	109	72.6%
3	Increased degree of Breathlessness	150	100%
4	Fever>38□ C	20	13.3%
5	Increased wheeze/crepitations	150	100%

Increased level of breathlessness and wheeze were the commonest symptom among patients with AECOPD(100%) followed by sputum volume(80%),and sputum purulence(72.6%)

TABLE 7: CATOGERISATION OF AECOPD PATIENTS AS PER ANTHOSIEN CRITERIA[15]

Type of exacerbation	no	%
Type 1	109	72.6
Type 2	11	7.3
Type 3	30	20

As per Anthosien criteria Type 1 exacerbation was the most common (72.6%) with all three cardinal symptoms followed by Type3 exacerbation(20%).

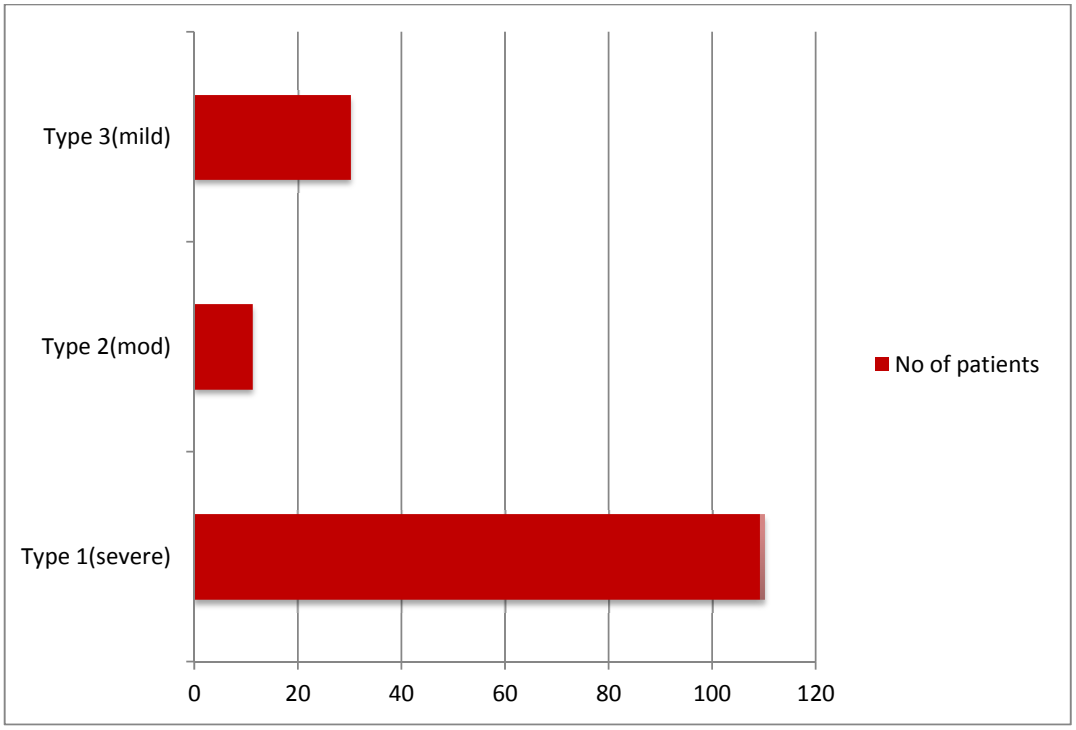


TABLE 8 : COMPLICATIONS ASSOCIATED WITH AECOPD PATIENTS (n=150)

S.no	Complication	No	%
1.	Cor pulmonale	11	7.3
2.	Respiratory failure type 1	12	8
3.	Respiratory failure type 2	4	2.6
4.	Bacteremia	9	6
	Total	36	24

Complications were observed in 36 (24%) patients among AECOPD patients among which Respiratory failure (8%) was the most common complication followed by Corpulmonale (7.3%) and Bacteremia (6%).Stable COPD study group was not associated with any complications.

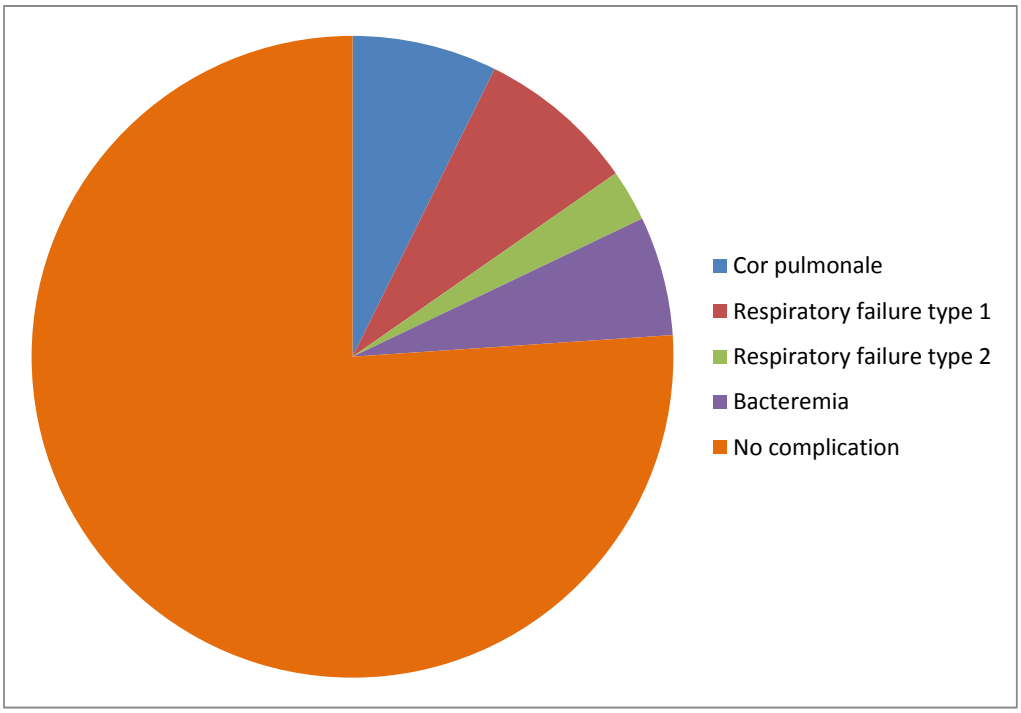


TABLE 9: CATEGORISATION OF PATIENTS BY GOLD[(1)] CRITERIA FOR SEVERITY AMONG STUDY GROUP

	AECOPD n=150		**Stable COPD n=50**	
Type	No	**%**	**No**	**%**
Mild	4	2.6	9	18
Moderate	86	57.3	41	82
Severe	60	40	-	-

Among AECOPD patients, 57.3% of patients had moderately severe condition followed by Severe type (40%).Among Stable COPD patients most common type was moderate type 41(82%) followed by Mild type 9(18%).

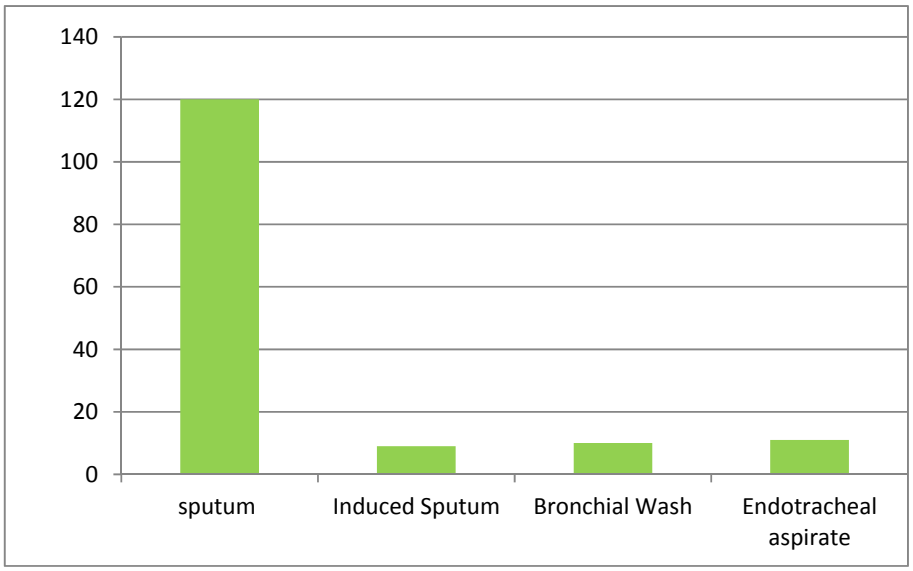
TABLE 10: DISTRIBUTION OF CULTURE POSITIVITY IN DIFFERENT SAMPLES

	Sample	**no (%)**	**Culture positive %**
RESPIRATORY SAMPLES	SPUTUM	119(79.3%)	77(64.7%)
	INDUCED SPUTUM	9 (6%)	8(88%)
	BRONCHIAL WASH	10(6.6%)	10(100%)
	ENDOTRACHEAL ASPIRATE	12(8%)	11(91.6%)
TOTAL		150	106(70.6%)
BLOOD	BLOOD	150	9(6%)

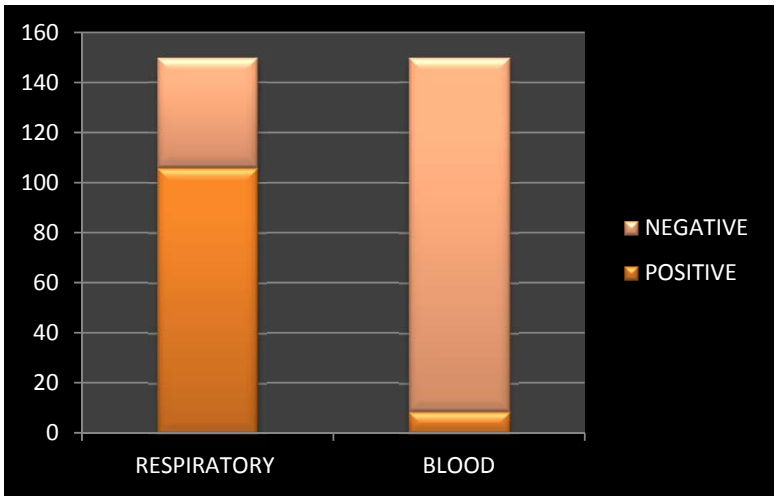
The most common sample obtained was Sputum(79.3%) in which culture positivity was 64.7%.Bronchial wash and Endotracheal aspirates had the highest

culture positivity rate of 100% and 91.6% respectively.Bacteremia was observed in 6% of patients.

DISTRIBUTION OF SAMPLE TYPES



DISTRIBUTION OF CULTURE POSITIVITY IN RESPIRATORY AND BLOOD SAMPLES



Among respiratory samples 106 (70.6%) patients had culture positivity.6% of the patients with AECOPD had Bacteremia.

TABLE 11: DISTRIBUTION OF PATHOGENS AMONG AECOPD PATIENTS FROM RESPIRATORY SAMPLES

Types and combination of pathogens isolated	Culture positive isolates n= 106	
	No	**%**
Monobacterial	102	96.2
Polybacterial	4	3.8
Total	106	100

Monobacterial growth was observed in 96.2%.Two or more organisms was isolated in 3.8% of cases.

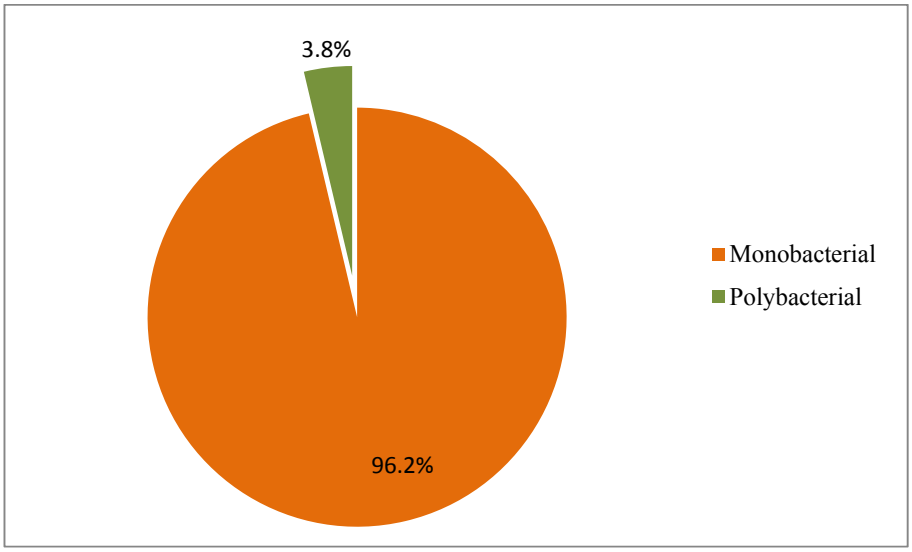


TABLE 12: DISTRIBUTION OF SINGLE BACTERIAL ISOLATES FROM RESPIRATORY AND BLOOD SAMPLES IN AECOPD CASES

Sample	Gram positive isolates		Gram negative isolates	
	No	%	No	%
Respiratory samples n=102	23	22.5	79	77.5
Blood samples n=9	4	44.5	5	55.5

Gram negative bacteria were isolated in 79(77.4%) patients while Gram positive bacteria were isolated in 23(22.5%) cases from respiratory samples.Gram negative bacteria were isolated in 5(55.5%) while Gram positive bacteria were isolated in 4(44.5%) cases from blood cultures.

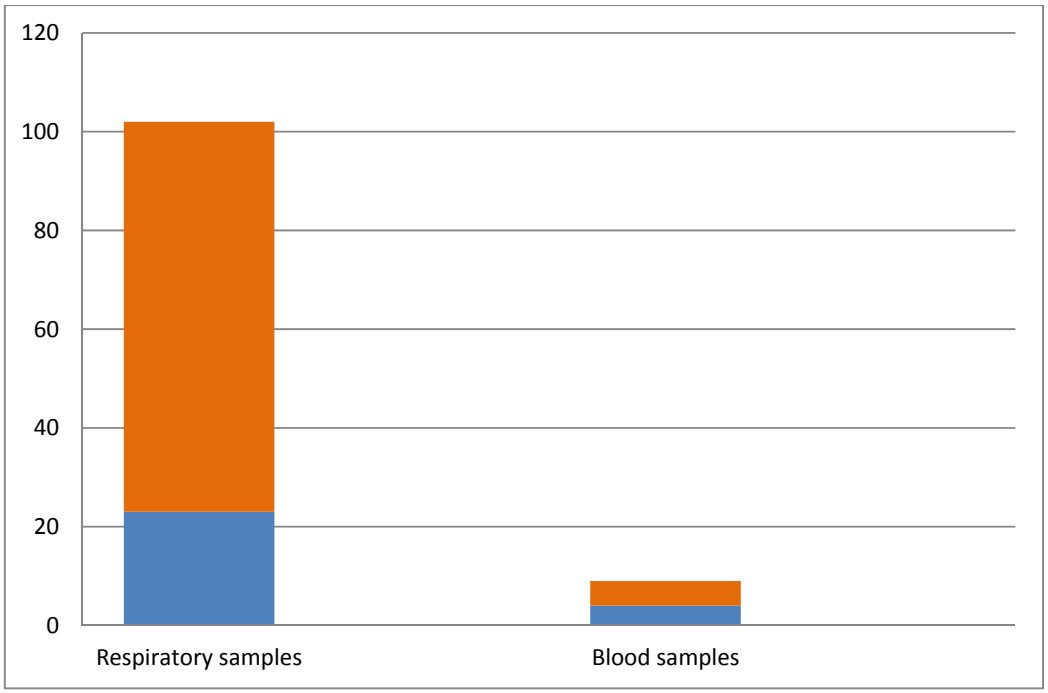


Table 13: ASSOCIATION OF SPUTUM PURULENCE WITH CULTURE POSITIVITY IN AECOPD PATIENTS

Type of sputum	Culture positivity	Culture negativity
Purulent Sputum n=109	75	4
Non purulent Sputum n=41	2	38

Culture positivity was significantly higher in patients with purulent sputum as compared to nonpurulent sputum. (P <0.001,Chi Square test)

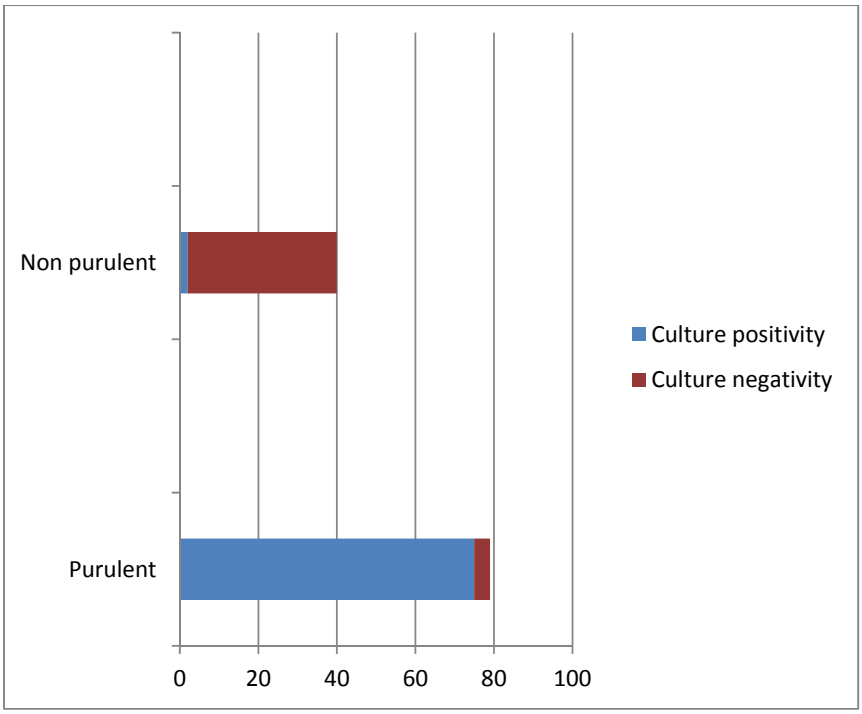


TABLE 14: **SPECIES DISTRIBUTION OF MONOBACTERIAL CULTURE POSITIVITY AMONG RESPIRATORY SAMPLES IN AECOPD PATIENTS (n=102)**

Sample	***S. aureus***	***S pneumoniae***	***K. pneumoniae***	***K. oxytoca***	***P. Aeruginosa***	***A. baumanii***	***M. catarrhalis***
Sputum	14	6	26	6	17	4	3
Induced Sputum	1	-	2	1	1	1	-
Bronchial Wash	-	-	3	-	3	4	-
Endotracheal aspirate	1	1	3	-	-	1	-
Total	16 (15.6%)	7 (6.8%)	34 (33.3%)	7 (6.8%)	24 (23.5%)	11 (10.7%)	3 (2.9%)

The most common pathogen isolated in sputum, induced sputum and endotracheal aspirate was *Klebsiella pneumoniae*(33.3%) followed by *Pseudomonas aeruginosa* 24(23.5%). This was statistically analysed by Chisquare test. P value was <0.001 which was significant.

SPECIES DISTRIBUTION OF MONOBACTERIAL CULTURE POSITIVITY AMONG RESPIRATORY SAMPLES IN AECOPD PATIENTS (n=102)

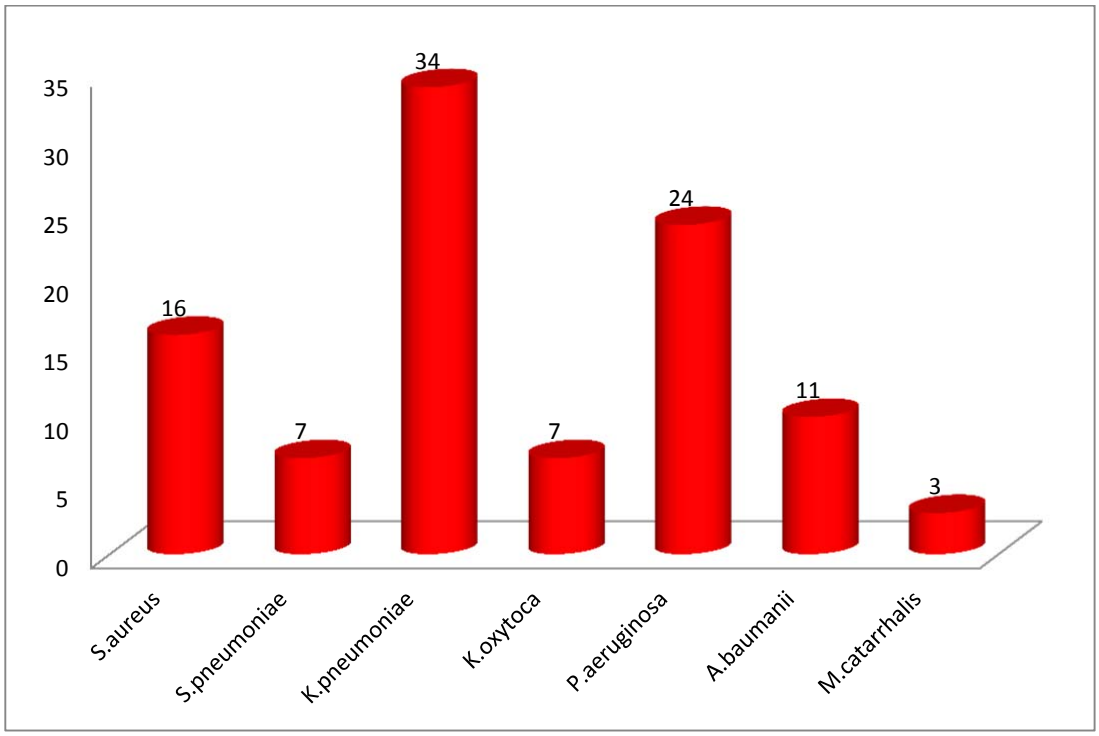


TABLE15: DISTRIBUTION OF MIXED INFECTION OF MICROBIAL ISOLATES AMONG RESPIRATORY SAMPLES

Sample	***Klebsiella oxytoca*** + ***Acinetobacter baumanii***	***Klebsiella pneumoniae*** + ***Pseudomonas aeruginosa***
Sputum	-	1
Endotracheal aspirate	1	2

Mixed infection was found in 3 endotracheal aspirate and a single sputum sample.

TABLE16: DISTRIBUTION OF ISOLATES AMONG VARIOUS CATEGORIES OF AECOPD FROM RESPIRATORY SAMPLES(n=110)

Organism isolated	Mild	Moderate	Severe
Staphylococcus aureus n=16	1(0.9%)	12(10.9%)	3(2.7%)
Streptococcus pneumoniae n=7	-	2(1.8%)	5(4.5%)
Klebsiella pneumoniae n=37	-	25(22.7%)	12(10.9%)
Klebsiella oxytoca n=8	-	6(5.5%)	2(1.8%)
Pseudomonas aeruginosa n=27	-	4(3.6%)	23(20.9%)
Acinetobacter baumanii n=12	-		12(10.9%)
Moraxella catarrhalis n=3	-	3(2.7%)	

In moderate type of COPD patients *Klebsiella pneumonie* contributed to 25(22.7%) followed by *Staphylococcus aureus* 12(10.9%).In severe type of COPD cases *Pseudomonas aeruginosa* contributed to 23(20.9%) followed by *Acinetobacter baumanii* 12(10.9%).This was statistically analysed by Chi square test with a P value of <0.001 which was significant. Nonfermenters were significantly isolated in Severe type.

DISTRIBUTION OF ISOLATES AMONG VARIOUS CATEGORIES OF AECOPD FROM RESPIRATORY SAMPLES

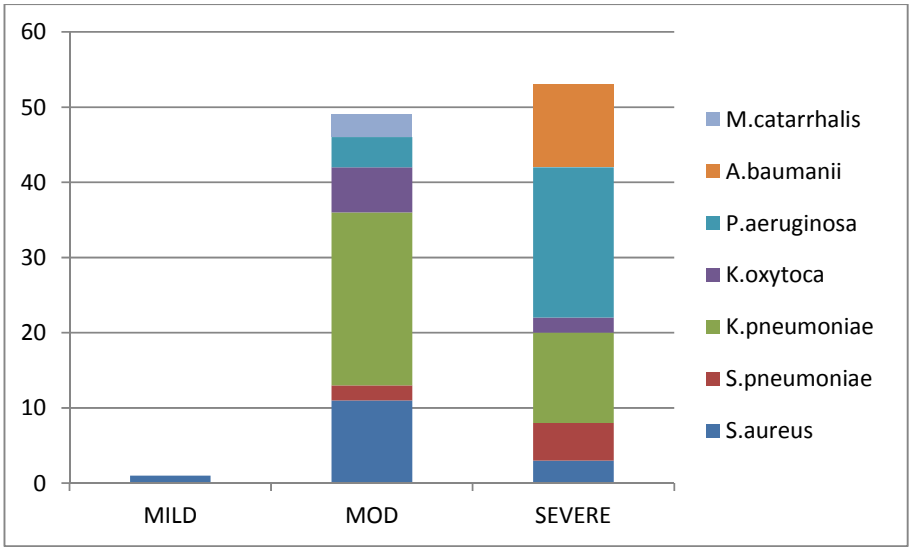


TABLE 17: DISTRIBUTION OF ETIOLOGICAL ISOLATES AMONG VARIOUS CATEGORIES OF AECOPD FROM BLOOD SAMPLES (n=9)

Severity	Mild	Moderate	Severe
Staphylococcus aureus	-	-	4(44.4 %)
Klebsiella pneumonia	-	-	4(44.4%)
Acinetobacter baumanii	-	-	1(11.2%)

All the 3 blood stream isolates were retrieved from severe type of COPD

TABLE 18:DISTRIBUTION OF ISOLATES IN STABLE COPD PATIENTS (n=50)

Type	*Klebsiella pneumoniae*		Normal flora	
	NO	%	NO	%
MILD	3	6	6	12
MODERATE	6	12	35	70
SEVERE	-	-	-	-

Klebsiella pneumoniae was the only isolate in stable COPD patients. It was isolated in 12% from moderate type category and 6% from mild type .In remaining patients normal flora was isolated.

TABLE 19: DISTRIBUTION OF *KLEBSIELLA PNEUMONIAE* ISOLATES AMONG AECOPD AND STABLE COPD PATIENTS FROM RESPIRATORY SAMPLES IN CULTURE POSITIVE SAMPLES:

	Klebsiella pneumoniae isolated	*Klebsiella pneumoniae not isolated*
AECOPD	37	73
STABLE COPD	9	0

Klebsiella pneumoniae isolated was significantly higher in AECOPD patients as compare to stable cases.This was analysed statistically by Chisquare test .P value was <0.001 which was significant.

TABLE 20: ANTIMICROBIAL SUSCEPTIBILITY OF GRAM POSITIVE COCCI FROM RESPIRATORY SAMPLES IN AECOPD PATIENTS BY KIRBY BAUER DISC DIFFUSION TEST:

Antibiotics µg	***Staphylococcus aureus* n=16**		***Streptococcus pneumoniae* n=7**	
	No	**%**	**NO**	%
Amikacin(30)	16	**100**	**NA**	NA
Penicillin(10 units)	8	**50**	**-**	**-**
Cefoxitin(30)	10	**62.5**	**NA**	**NA**
Ciprofloxacin(5)	12	**75**	**-**	**-**
Chloramphenicol(30)	-	**-**	**7**	100
Cotrimoxazole(1.25/23.75)	13	**81**	**7**	100
Erythromycin(15)	12	**75**	**7**	100
Ofloxacin(5)	-	**-**	**7**	100
Oxacillin(1)	-	**-**	**5**	71.4
Tetracycline(30)	13	81	7	100
Vancomycin	NA		7	100

NA-These antibiotics are not recommended by CLSI guidelines for that particular group of organisms.

All *Staphylococcus aureus* showed 100% sensitivity to Amikacin. All *Streptococcus pneumoniae* isolates from respiratory samples were 100% sensitive to Erythromycin, Cotrimoxazole, Ofloxacin, Tetracycline and Vancomycin Two of the *Streptococcus pneumoniae* isolates from the respiratory samples showed resistance to Oxacillin.

TABLE 21:ANTIMICROBIAL SUSCEPTIBILITY OF GRAM NEGATIVE COCCI FROM RESPIRATORY SAMPLES IN AECOPD PATIENTS BY KIRBY BAUER DISC DIFFUSION TEST:

Antibiotics µg	***Moraxella catarrhalis* n=3**	
	No	%
Amikacin(30)	NA	NA
Ampicillin(10)	2	66.6
Penicillin(10 units)	NA	NA
Amoxycillin-Clavulanic Acid(20/10)	100	100
Cefoxitin(30)	NA	NA
Ciprofloxacin(5)	3	100
Cotrimoxazole(1.25/23.75)	2	66.6
Erythromycin(15)	3	100
Tetracycline(30)	2	66.6

NA-These antibiotics are not recommended by CLSI guidelines for that particular group of organisms.

TABLE 22: ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROBACTERIACEAE ISOLATES FROM RESPIRATORY SAMPLES IN AECOPD PATIENTS BY KIRBY BAUER DISC DIFFUSION TEST:

Antibiotics µg	***Klebsiella pneumoniae* n=37**		***Klebsiella oxytoca* n=8**	
	NO	**%**	**NO**	**%**
Amikacin(30)	33	89.1	8	100
Cefotaxime(30)	14	37.8	5	62.5
Cefpodoxime	14	37.8	5	62.5
Cotrimoxazole(1.25/23.75)	21	56.7	5	62.5
Ciprofloxacin(5)	21	56.7	7	87.5
Gentamicin(10)	30	81	7	87.5
Imipenem(10)	34	91.8	7	87.5
Piperacillin – Tazobactam(100/10)	30	81	7	87.5
Tetracycline(30)	37	100	8	100

MDR- Multi drug resistance

In *Klebsiella pneumoniae* resistance to Cefotaxime, Ciprofloxacin and Cotrimoxazole were shown in 23(62.2%),16(43.3%) and 17(43.3%) isolates respectively. Third generation Cephalosporin resistance pattern were further phenotypically tested for ESBL production. Multi drug resistance strains (Cefotaxime, Ciprofloxacin and Cotrimoxazole) was noted in 11(29.7%) of isolates. Resistance to Imipenem was noted in 3(8.2%)isolates .

In *Klebsiella oxytoca* resistance to Cefotaxime ,Ciprofloxacin and Cotrimoxazole were shown in 5(37.5%) ,7(12.5%), and 5 (37.5%) isolates respectively. Third generation Cephalosporin resistance pattern were further phenotypically tested for ESBL production. Resistance to Imipenem was noted in 1(12.5%)isolates .MDR strains was noted in one(12.5%) isolate.

TABLE 23:ANTIMICROBIAL SUSCEPTIBILITY OF *KLEBSIELLA PNEUMONIAE* FROM RESPIRATORY SAMPLES IN STABLE COPD PATIENTS BY KIRBY BAUER DISC DIFFUSION TEST:

Antibiotics	***Klebsiella pneumoniae*** **n=9**	
	NO	**%**
Amikacin(30)	9	100
Cefotaxime(30)	9	100
Cotrimoxazole(1.25/23.75)	7	77.8
Ciprofloxacin(5)	9	100
Gentamicin(10)	9	100
Imipenem(10)	9	100
Piperacillin – Tazobactam(100/10)	9	100
Tetracycline(30)	9	100

Klebsiella pneumoniae was the only bacteria isolated from cases of Stable COPD patients.2 isolates (22.2%) were resistant to Cotrimoxazole .All isolates were 100% susceptible to Amikacin ,Cefotaxime, Ciprofloxacin, Gentamicin , Piperacillin tazobactam combination.

TABLE 24: ANTIMICROBIAL SUSCEPTIBILITY OF GRAM NEGATIVE NON – FERMENTOR ISOLATES FROM RESPIRATORY SAMPLES IN AECOPD PATIENTS BY KIRBY BAUER DISC DIFFUSION TEST:

Antibiotics	*Pseudomonas aeruginosa* n=27		*Acinetobacter baumanii* n=12	
	NO	%	NO	%
Amikacin	24	88.8	9	75
Ceftazidime	14	51.3	5	41.7
Cotrimoxazole	NA		8	66.7
Ciprofloxacin	23	85.1	8	66.7
Gentamicin	21	77.8	7	58.3
Imipenem	26	96.3	10	83.3
Piperacillin – Tazobactam	24	88.8	9	75

13 (48.2%) isolates of *Pseudomonas aeruginosa* were resistant to Ceftazidime in and 7(58.3%) Isolates of *Acinetobacter baumanii* were resistant to Ceftazidime. Imipenem resistance was seen in one (3.7%) isolate of *Pseudomonas aeruginosa* and two(16.7%) isolates of *Acinetobacter baumanii*.MDR strains were noted in 1(3.7%) *Pseudomonas aeruginosa* isolate and 3(25%) *Acinetobacter baumanii* isolate.

TABLE 25:ANTIMICROBIAL SUSCEPTIBILITY OF ETIOLOGICAL ISOLATES FROM BLOOD SAMPLES IN AECOPD PATIENTS BY KIRBY BAUER DISC DIFFUSION TEST:

Antibiotics µg	***Staphylococcus aureus* n=4**		***Klebsiella pneumoniae* n=4**		***Acinetobacter baumanii* n=1**	
	NO	**NO**	**%**	%	NO	%
Amikacin(30)	4	100	3	75	1	100
Ampicillin(10)	2	50	-	-	-	-
Penicillin(10 units)	2	50	-	-	NA	NA
Cefoxitin(30)	2	50	NA	NA	NA	NA
Ciprofloxacin(5)	4	100	2	50	1	100
Ceftazidime(30)	-	-	-	-	0	
Cefotaxime(30)	-	-	1		-	-
Chloramphenicol(30)	-	-	4	100	1	100
Cotrimoxazole(1.25/23.75)	4	100	0		1	100
Erythromycin(15)	4	100	-	-	-	-
Gentamicin(10)	-	-	3	75	1	100
Imipenem(10)	NA	NA	4	100		100
Piperacillin – Tazobactam(100/10)	NA	NA	3	75	1	100
Tetracycline(30)	-	-	4	100	1	100

All the *Staphylococcus aureus* showed 100% sensitivity to Amikacin, Ciprofloxacin, Erythromycin, and Cotrimoxazole. Among them two MRSA were isolated. Among *Klebsiella pneumoniae* all the isolates showed 100% sensitivity to Chloramphenicol, Imipenem and Tetracycline. Among them 3 of the isolates were ESBL producers. All *Acinetobacter baumanii* isolates showed 100% sensitivity to all drugs.

TABLE 26: DETERMINATION OF ESBL ISOLATES FROM RESPIRATORY AND BLOOD SAMPLES IN AECOPD PATIENTS

S. No.	Samples	Isolates	Screening Test		DDST		PCDDT	
			Positive	%	No.	%	No.	%
1.	Respiratory samples n=45	*Klebsiella pneumoniae*	16	35.5	18	40%	18	40%
		Klebsiella oxytoca	2	4.5				
2.	Blood samples n=4	*Klebsiella pneumoniae*	3	75	3	75%	3	75%

DDST **–** Double disk diffusion synergy test

PCDDT – Phenotypic Confirmatory disk diffusion test

TABLE 27 : DETERMINATION OF IMIPENEM RESISTANT ISOLATES FROM RESPIRATORY SAMPLES OF AECOPD

No.of isolates n=84	Imipenem screening –disc diffusion method		Meropenem MIC Macrobroth dilution method	
	S	R	MIC value	Interpretation
Klebsiella pneumoniae n=37	34	3	64	Resistant
Klebsiella oxytoca n=8	7	1	32	Resistant
Pseudomonas aeruginosa n=27	23	1	64	Resistant
Acinetobacter baumanii n=12	10	2	64	Resistant

The MIC value of all the seven isolates of Gram negative bacilli for Meropenem were in resistant range.

Table 28: DETERMINATION OF METALLOBETALACTAMASE PRODUCTION BY DDST AMONG AECOPD PATIENTS

Respiratory samples	**MBL PRODUCTION**	
	Positive	**Negative**
Klebsiella pneumoniae n=3	Nil	3
Klebsiella oxytoca n=1	Nil	1
Pseudomonas aeruginosa *n*=1	Nil	1
Acinetobacter baumanii n=2	Nil	2

DDST :Imipenem- EDTA double disc synergy test

None of the isolates were Metallobetalactamase producers.

TABLE 29: DETERMINATION OF Amp C PRODUCTION AMONG ISOLATES FROM RESPIRATORY SAMPLES OF AECOPD PATIENTS

Isolate	**Screening test positive**	**AmpC disc confirmatory test positive**
Klebsiella pneumoniae *n=3*	Nil	-
Klebsiella oxytoca *n=1*	Nil	-
Pseudomonas aeruginosa *n=3*	Nil	-
Acinetobacter baumanii *n=2*	1	1

According to AmpC production definition Only one isolate among 12 *Acinetobacter baumanii* isolates from endotracheal aspirate were AmpC producer(8.%).

TABLE 30: DETERMINATION OF CARBAPENAMASE PRODUCTION AMONG IMIPENEM RESISTANT ISOLATES FROM RESPIRATORY ISOLATES IN AECOPD PATIENTS BY MODIFIED HODGE TEST(MHT) n=7

Isolates	NO	Positive	%
Klebsiella pneumoniae	3	2	28.5
Klebsiella oxytoca	1	-	
Pseudomonas aeruginosa	1	-	
Acinetobacter baumanii	2	1	14.3

Imipenem resistance was taken as indicator for Carbapenemase production. Among 7 Imipenem resistant isolates 2(28.5%) *Klebsiella pneumonia* isolates and 1 (14.3%) *Acinetobacter baumanii* isolate were Carbapenemase producing organism which was comfirmed by MHT.

Table 31: DETERMINATION OF METHICILLIN RESISTANCE AND VANCOMYCIN MIC OF *STAPHYLOCOCCUS AUREUS* BY MACROBROTH DILUTION METHOD ISOLATES FROM RESPIRATORY SAMPLES OF AECOPD PATIENTS(N=16)

Cefoxitin screening –disc diffusion method			MIC of Vancomycin	Result
Sensitive	Resistant	%		
10	6	37.5	≤2µg/ml	Sensitive

Among 16 isolates 6(37.5%) isolates were Methicillin resistant. All MRSA isolates had MIC ≤2µg/ml for Vancomycin and therefore considered sensitive.

Table 32 :DETERMINATION OF DRUG RESISTANT STREPTOCOCCUS PNEUMONIAE ISOLATES FROM RESPIRATORY SAMPLES OF AECOPD (n=7)

Oxacillin screening –disc diffusion method			Penicillin MIC by E test	
S	R	%	MIC value	Interpretation
5	2	28.5	0.094µg/ml 0.094µg/ml	Intermediate resistance Intermediate resistance

The MIC value of all the two isolates of *Streptococcus pneumoniae* were in intermediate range.

TABLE 33: DISTRIBUTION OF RESISTANT ISOLATES AS PER AECOPD SEVERITY IN RESPIRATORY SAMPLES

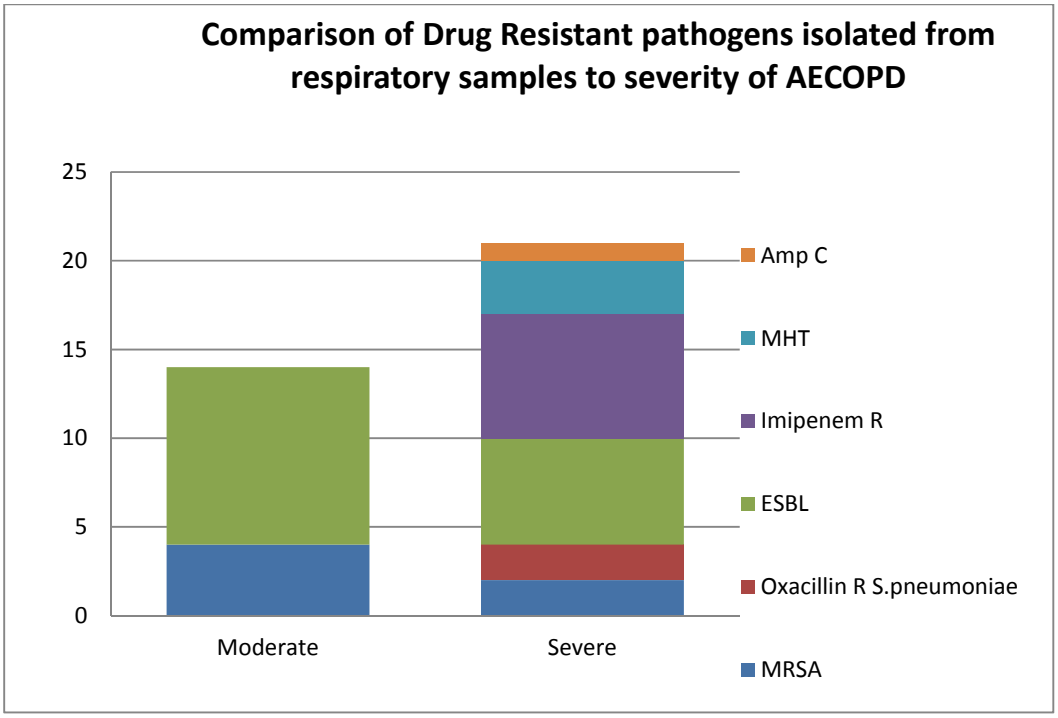
Severity	MRSA n=6	Pencillin resistant Streptococcus pneumoniae n=7	ESBL n=18	Imipenem Resistant n=4	MHT n=2	AmpC	Imipenem resistant n=3	MHT n=1	AmpC n=1
			Enterobacteriaceae				Non-Enterobacteriaceae n=39		
Mild		-	-	-	Nil	Nil	-	Nil	-
Mod	4 66.7%	-	12 66.7%	-	-	-	-	-	-
Severe	2 33.3%	2 100%	6 33.3%	4 100%	2 100%	-	3 100%	1 100%	1 100%

MHT- Modified Hodge test

MRSA- Methicillin resistant *Staphylococcus aureus*

ESBL-Extended spectrum betalactamase

This was analysed statistically by Chisquare test which showed a P value of <0.001 . It was significant. So Presence of MRSA and ESBL isolates were significantly higher in Moderate AECOPD patients (66.6%, 66.6% , respectively) than in severe group (33.3%,33.3%). Penicillin resistant *Streptococcus pneumoniae*, Imipenem resistance, Carbapenamase producing isolates(Modified hodge test positive),Amp C producing strain were significantly higher in severe AECOPD patients (100%).



COLOUR PLATES

FIGURE 1 : POSTERO ANTERIOR CHEST X RAY OF CHRONIC BRONCHITIS

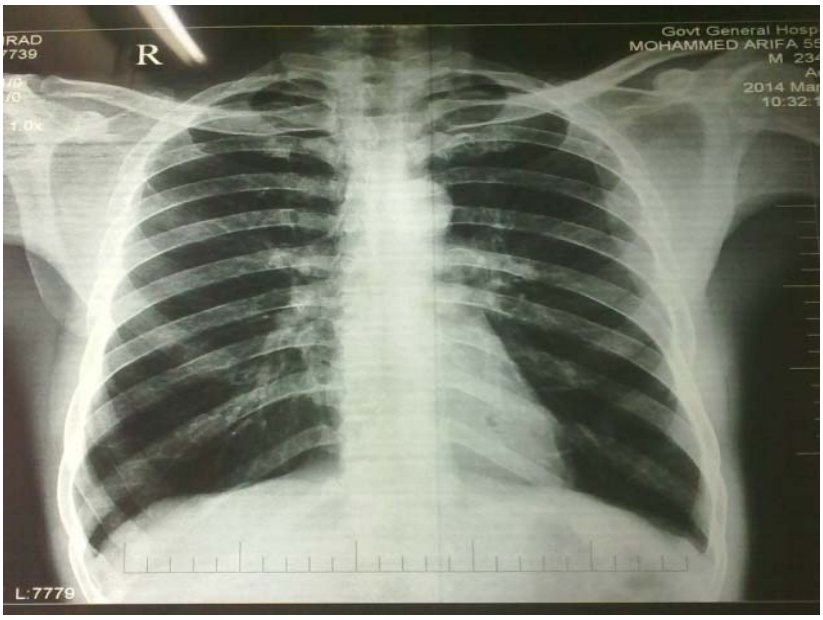


FIGURE 2: SPIROMETER

FIGURE 3: SPUTUM PROCESSING

FIGURE 4: SPUTUM QUANTIFICATION

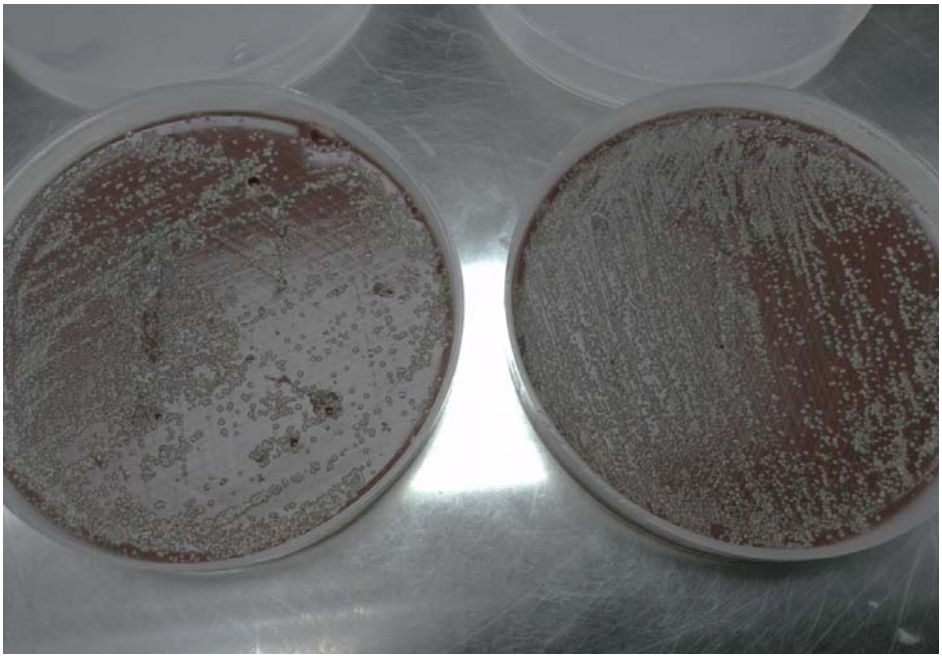
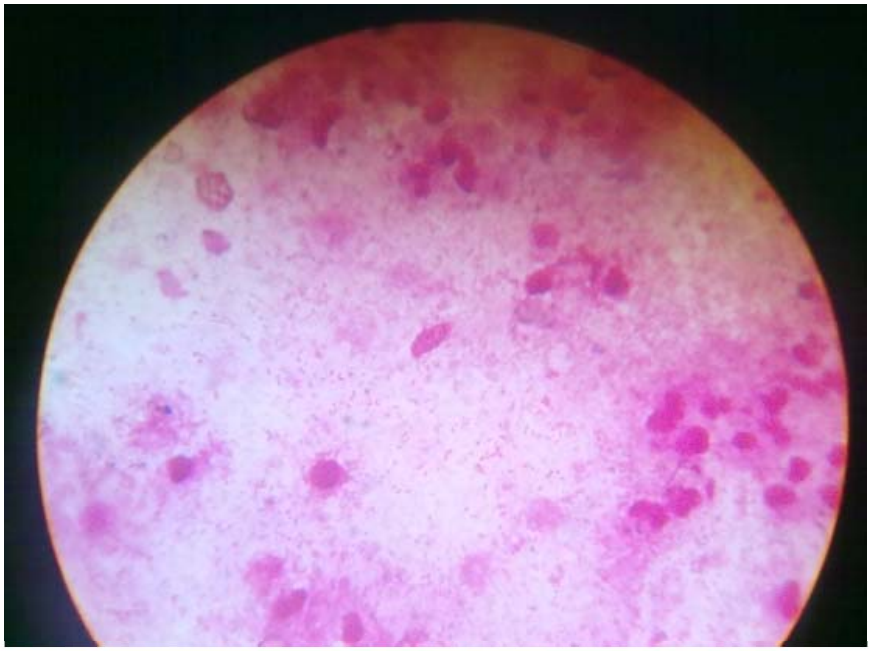


FIGURE 5 & 6 : DIRECT GRAM STAIN FROM SPUTUM SAMPLE SHOWS PLENTY OF PUS CELLS & GRAM NEGATIVE BACILLI



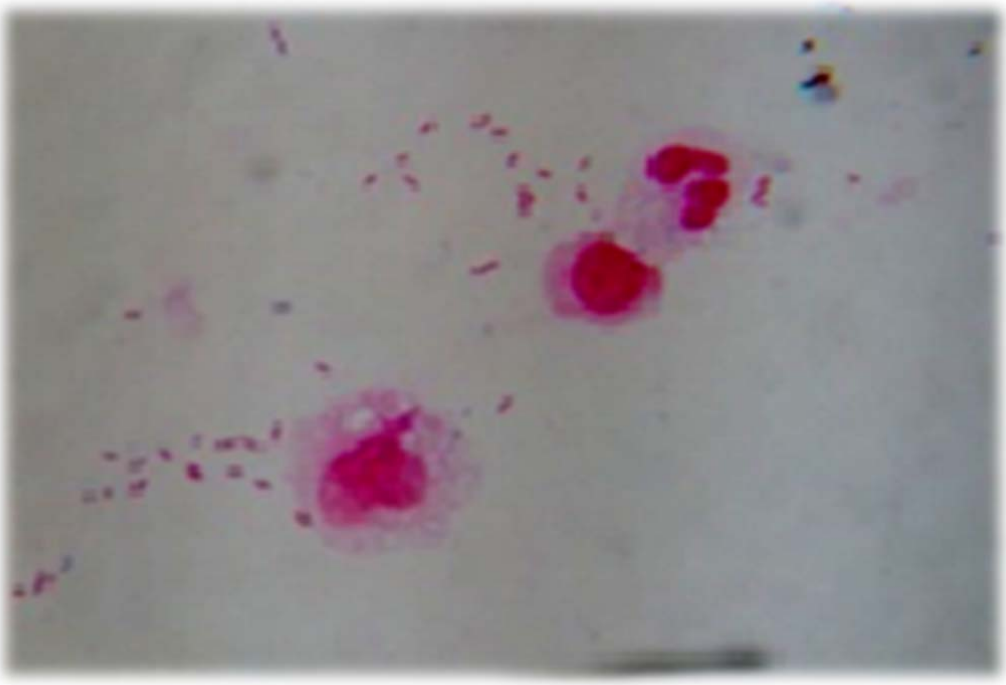


FIGURE 7: LACTOSE FERMENTING COLONIES ON MacConkey PLATE

FIGURE 8:PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST (PCDDT) FOR ESBL PRODUCTION

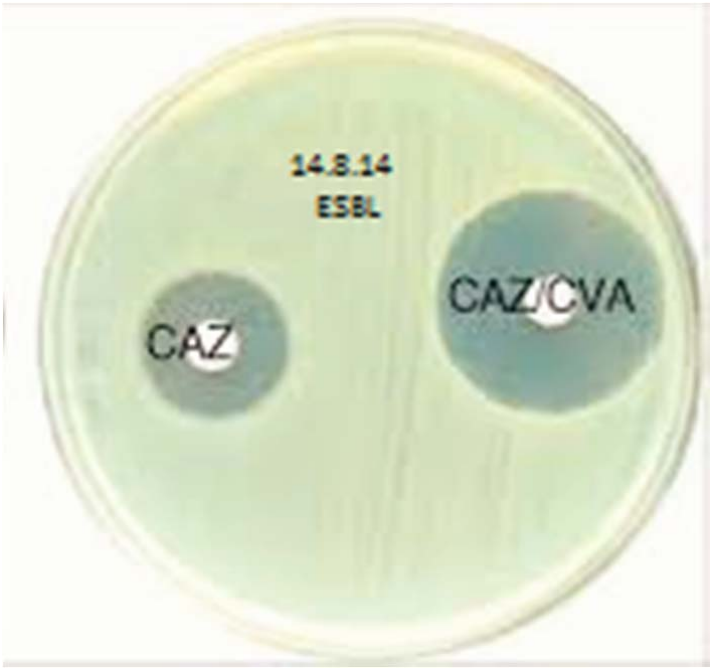


FIGURE 9 : DOUBLE DISC SYNERGY TEST (DDST)FOR ESBL PRODUCTION

FIGURE 10:
MODIFIED HODGE TEST

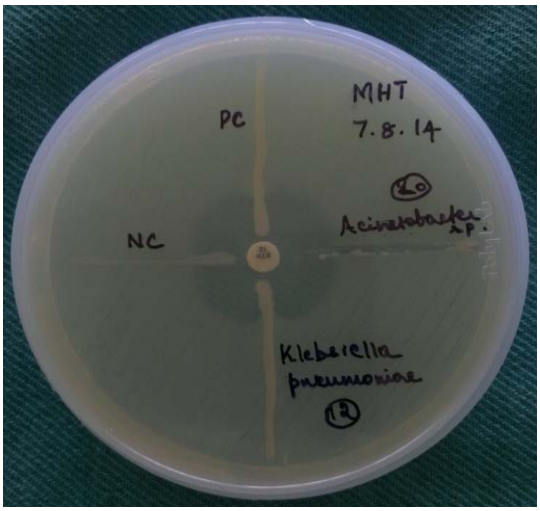


FIGURE 11: AmpC PRODUCTION BY DISC TEST

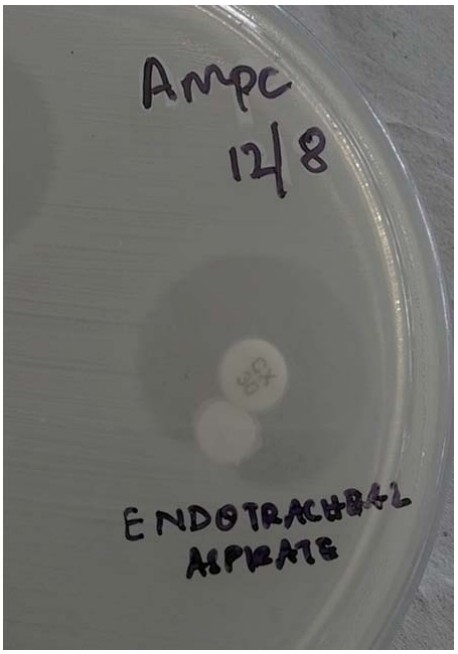


FIGURE 12-15:MIC DETERMINATION BY MACROBROTH DILUTION METHOD of IMIPENEM

FIGURE 16 : DIRECT GRAM STAIN OF SPUTUM SAMPLE SHOWS PLENTY OF PUSCELLS AND GRAM POSITIVE COCCI IN PAIRS

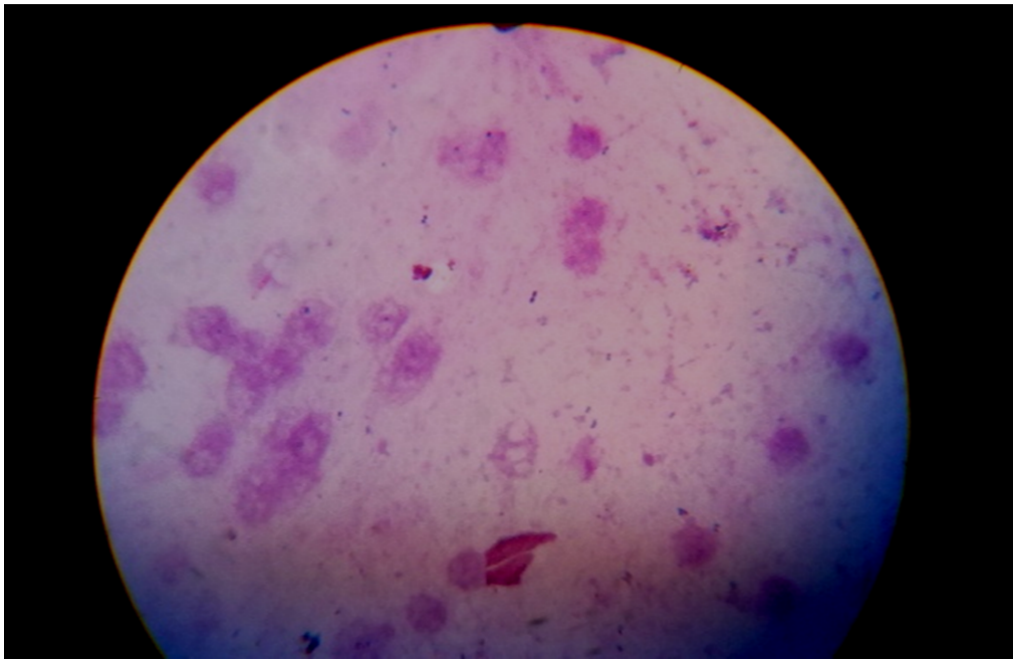


FIGURE 17: GRAM STAIN FROM CULTURE SHOWING GRAM POSITIVE COCCI IN PAIRS

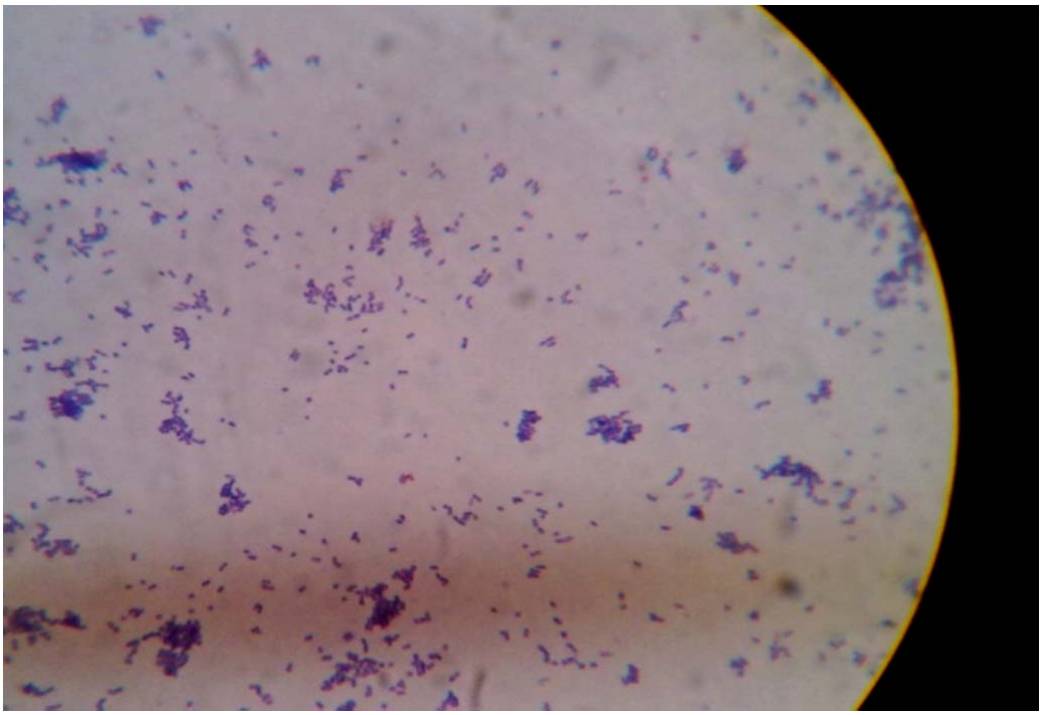


FIGURE 18: 5% SHEEP BLOOD AGAR SHOWS ALPHA HAEMOLYTIC COLONIES

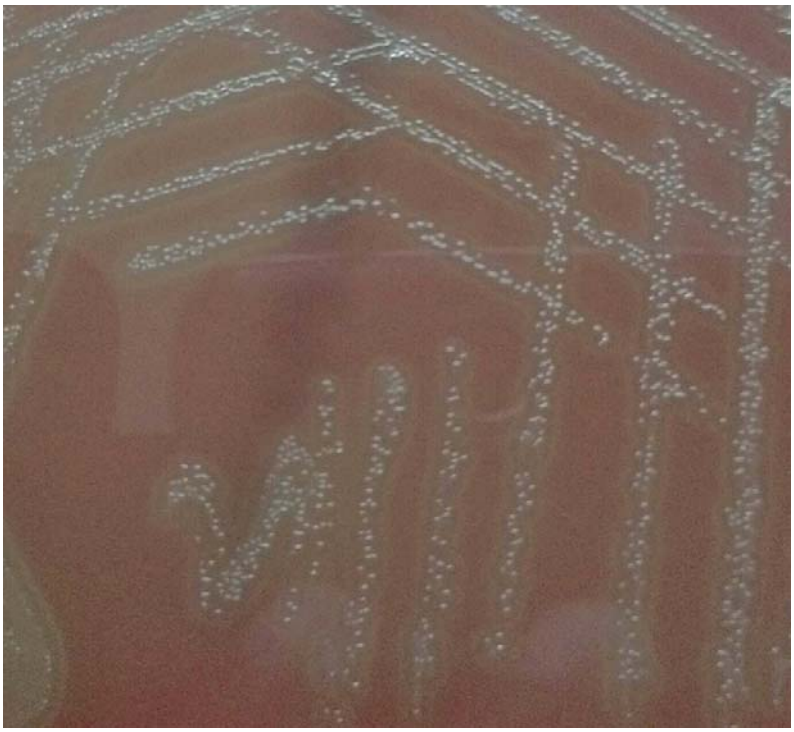


FIGURE19: OPTOCHIN SENSITIVITY OF STREPTOCOCCUS PNEUMONIAE

FIGURE 20& 21:ANTIMICROBIAL SUCEPTIBILITY OF *STREPTOCOCCUS PNEUMONIAE*

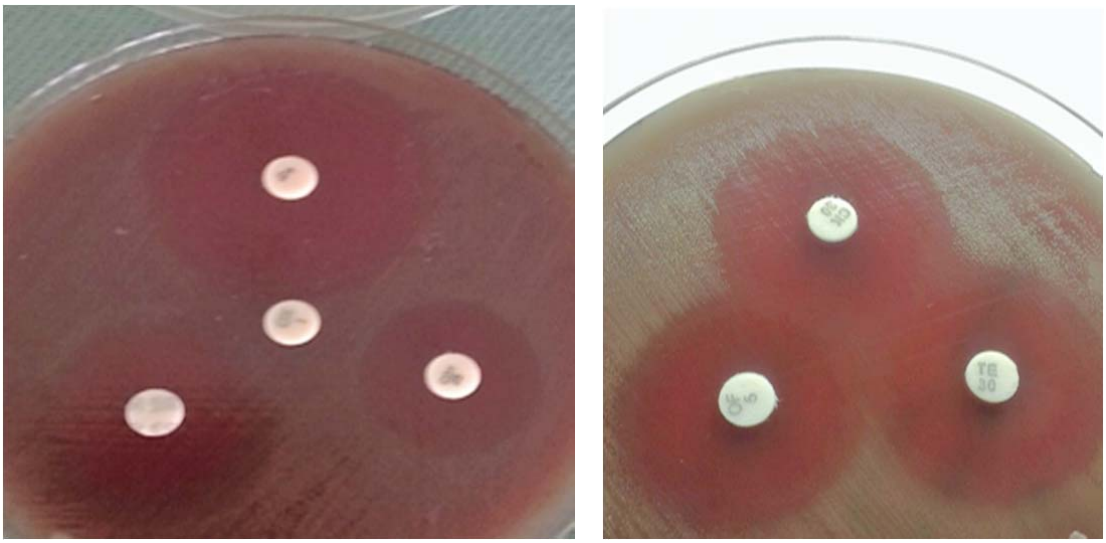


FIGURE 23 & 24: MIC DETERMINATION OF PENICILLIN BY E STRIP FOR *STREPTOCOCCUS PNEUMONIAE*

FIGRE 25 : DIRECT GRAM STAIN FROM SPUTUM SHOWS PLENTY OF PUS CELLS &GRAM POSITIVE COCCI IN CLUSTERS

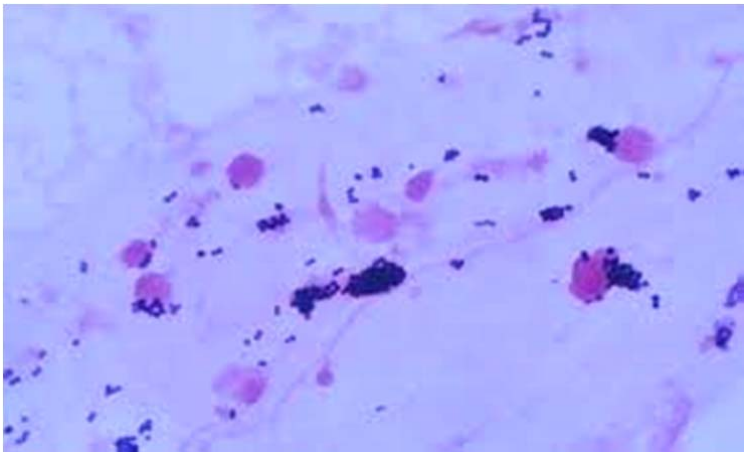


FIGURE 26: ANTIBIOGRAM OF STAPHYLOCOCCUS AUREUS SHOWING METHICILLIN RESISTANC BY CEFOXITIN DISC

FIGURE 27: MIC DETERMINATION BY MACROBROTHDILUTION METHOD OF VANCOMYCIN FOR STAPHYLOCOCCUS AUREUS

DISCUSSION

DISCUSSION

This Cross sectional study was conducted at the Institute of Microbiology, Madras Medical College in association with Departments of InternalMedicine, Thoracic Medicine, Intensive Medical Care Unit at the Rajiv Gandhi Government General Hospital, Chennai. The study population consisted of 150 patients with AECOPD and 50 patients with Stable COPD .The Stable COPD patients were included as a control population to compare the pattern of microorganisms isolated. Patients were selected according to GOLD[14] criteria .

The study population included in the AECOPD category were in the age group of 40-90 years .The majority of AECOPD patients were in the age group of 61-70 years (30%) (Table 1).In the Stable COPD category, 42% patients were in the age group of 61-70 years (Table 2).This can be explained by the fact that COPD has the highest prevalence after fifth and sixth decade of life . As age advances ,the physiological decrease in lung function is accentuated by the cumulative damage done by smoking and other co morbid conditions.[67] This study observation corresponds to studies of Alladi Mohan *et al*, N Arora *et al* and Pallavi Torka *et al*. [68-70]

There was a predominance of males over females in both AECOPD (82.7% and 17.3%) and in stable COPD (84% and 16%) patients(Table 1,Table 2). This can be explained by the fact that men have pronounced smoking habits and are exposed more to outside environment as compared to females. This was similar to the study done by Chawla k et al. [75]

Smoking leads to decreased mucociliary clearance and innate immunity thereby leading to increased bacterial colonization that can give rise to increased airway inflammation and thus exacerbation.[71]In the present study smoking was associated with AECOPD in 81.3% of cases and 84% in Stable COPD cases(Table 3) followed by Biogas inhalation (13.3%) in AECOPD and 14% in stable COPD which was associated with female patients. This was similar to a study done by Jindal et al. who stated that smoking is associated with COPD in 80-85% of cases.[67] A study by Salvi et al. also states that indoor air pollution from burning biomass fuels is associated with an increased risk of COPD in developing countries.[105]Occupation as a risk factor of COPD was found only in 0.6% of patients in AECOPD and 2% in stable COPD.(Table 4). A study done by Piera Boschetto et al states that approximately 15% of COPD is occupation related[103] which is quite high as compared to this study.

Most common clinical diagnosis in both AECOPD and stable COPD patients was Chronic Bronchitis in (95.3% and 94%) respectively(Table 5). American Lung Association Lung Disease 2013 has estimated a higher prevalence of chronic bronchitis as compared to emphysema.[106]

The study group were selected based on Spirometric studies (GOLD criteria)[14].As per this criteria the most common type was moderate type (57.3%) followed by severe type (40%) in AECOPD patients. Karin et al. reported the severity of COPD as 47% (severe)and 32% (moderate). [81]Among Stable COPD patients most

common type was moderate type (82%) followed by Mild type (18%).No Severe type of COPD were detected in stable patients.(Table 9)

The AECOPD was further categorised as per Anthosien criteria[15].

Anthosien criteria is based on 3 cardinal symptoms -increased breathlessness, Increased volume of sputum and Increased purulence of sputum among which increase sputum purulence was more important for bacterial isolation.[15]

According to these criteria Increased level of breathlessness was the commonest symptom in AECOPD (100%).Next most common symptom was increased in sputum volume (80%), and sputum purulence (72.6%)(Table 6). This is in accordance with the study of N. Arora *et al* where 100% of patients had increase in sputum volume and 98.28% had various grades of dyspnoea. [68]

According to this criteria Type 1 exacerbation was the commonest (72.6%) type of AECOPD with all three cardinal symptoms followed by Type3 exacerbation (20%). (Table 7).

Complications were observed in 24% patients among AECOPD patients among which Respiratory failure was the most common complication(8%) followed by Corpulmonale (7.3%) and Bacteremia (6%) (Table 8) .These observations were corresponding to a study done by Alladi mohan et al. who reported Cor pulmonale in 10% and Type I Respiratory failure in 8% of patients.[74]Stable COPD study group was not associated with any complication .

The most common sample collected and processed from patients with AECOPD was Sputum (79.3%) followed by Endotracheal aspirate (8%). In addition ,Bronchial wash 6.6% and Induced sputum 6% were collected and processed . (Table10)

Out of 150 samples , 70.6% showed culture positivity and 29.4% (Table 10) showed culture negativity. Bronchial wash and Endotracheal aspirate had the highest culture positivity rate of 100% and 91.6% respectively followed by sputum(64.7%).(Table 10). Alamoudi OS et al. obtained growth in 69.8% of sputum samples which is corresponding with our study. [76] A similar observation was made by Arora et al. in which culture was positive in 72% cases among all respiratory samples[68].

The correlation of Sputum purulence with culture positivity was found to be statistically significant with a P value <0.001(Table 13). This correlates with the study done by Stockley RA et al., Soler et al and Chawla K et al. who reported a culture positivity among 84% ,84% and 56% respectively from purulent sputum samples from AECOPD patients. This proves that purulent sputum is the surrogate marker of bacterial infection as they yield positive bacterial cultures as compared to non purulent sputum.[72,73]

A Single organism was identified in 96.2% while poly bacterial growth was observed in 3.8% (Table 11).

Gerard rakesh et al. in their study on bacterial agents in AECOPD revealed 37% of monobacterial isolates and 5% of polybacterial isolates.[91]

In a study done by Chawla k et al.a single organism was isolated in most of the samples 92.85% and growth of two organisms was isolated in 7.14% cases. This was corresponding to the present study. [75]

The commonest organism in the respiratory samples in AECOPD patients were Gram negative bacteria (77.4%) as compared to Gram positive bacteria (22.5%)(Table 12). Among Gram negative organisms, *Klebsiella pneumoniae* 33.3% was the most commonly and significantly isolated organism(*P<0.001)* followed by *Pseudomonas aeruginosa* 23.5% , *Klebsiella oxytoca* 6.8%, *Acinetobacter baumanii* 10.7% and *Moraxella catarrhalis* 2.9% (Table14).Among Gram positive organisms, *Staphylococcus aureus* was most commonly isolated in 15.6% cases and *Streptococcus pneumoniae* in 6.8% of cases.

This is similar to the findings of Pradhan K .C *et al* who reported *Klebsiella pneumoniae* as the predominant organism (40%)followed by *Staphylococcus aureus (*26%) and *Pseudomonas aeruginosa (*13%). [82,83]

In a study done by Chawla K et al. *Pseudomonas aeruginosa* was the predominant isolate (25.92%) amongst the hospitalized patients followed by *Streptococcus pneumoniae* and *Acinetobacter* spp (18.51% each), *Klebsiella spp.* and *Moraxella catarrhalis* (14.80% each).[75]

Madhavi et al. observed that the *commonest isolate was Klebsiella pneumoniae (59%),* followed by *Pseudomonas aeruginosa 15%, Staphylococcus*

aureus 13.6%, Streptococcus pneumoniae 6.8% among AECOPD patients.[84]This was also corresponding to this study observation.

In a study done by Karien H. et.al. at University Hospital Maastricht in Netherlands most frequently isolated microorganisms were *Haemophilus influenzae* (45%) and *Streptococcus pneumoniae* (27%). Other pathogens isolated were *Pseudomonas aeruginosa* (15%), *Moraxella catarrhalis* (6%) and *Klebsiella pneumoniae* (5%). [81]

A study done by Eller jorg et al. stated that the predominant organism causing AECOPD was *Streptococcus pneumoniae , non typable Haemophilus influenzae* and *Moraxella catarrhalis* .[78]

Organisms isolated in western countries are different from our country. In contrast to Western literature , review of Indian literature shows no isolates of *Haemophilus influenzae* in AECOPD patients. This possible change in etiologic pattern may be due to advent of newer antibiotics with their indiscriminate usage, local practices of antibiotic usage or this might be due to the reason that the frequency of infection resulting in AECOPD by various microorganisms varies from one geographical area to another as our country has a wide climatic variation, small houses and high levels of indoor pollution. [85]

On analysis of the distribution of etiologic agents among various category of AECOPD it was found that in moderate type of COPD patients *Klebsiella pneumonia* contributed to 22.7% followed by *Staphylococcus aureus* 10.9%.In severe type

Pseudomonas aeruginosa contributed to 20.9% followed by *Acinetobacter baumanii* 10.9%(Table 16). Thus Non fermenters were significantly isolated in Severe type and *Klebsiella pneumoniae* from Moderate type with a significant *P value of <0.001.*

The present study observations corresponds to the study done by Eller jorg et al. which states that prevalence of *Pseudomonas* and *Acinetobacter* increases in patients with declining lung function[78].Similarly a study done by N.Roche et al. in 2007 reports that 8.5% of *Pseudomonas* sp were from patients with severe COPD[79].

Niederman micheal s.et al have mentioned in their study that Gram negative bacteria was the commonest bacteria isolated [80]. This study also corresponds with our findings.

Mixed infection among AECOPD patients was found in 3.8% of culture positive cases which was observed in three endotracheal aspirate and a single sputum sample.(Table 11).This polymicrobial infection was seen with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* followed by *Klebsiella oxytoca* and *Acinetobacter baumanii*. Gerard Rakesh et al. reported mixed infection in two cases of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and 3 cases of *Klebsiella pneumoniae* and *Staphylococcus aureus* which was similar to our findings [91] .

In stable COPD patients, culture positivity was 18% (Table 19). *Klebsiella pneumoniae* was the only potential pathogen isolated in 18% of these cases. In all other patients normal flora 82% were isolated. . *Klebsiella pneumoniae* was

isolated from 12% of moderate type and 6% of mild type .(Table 18) This was similar to study done by Mc Hardy et al. and Zalacain et al. who stated in their study that approximately 20–30% of patients with COPD have positive sputum bacterial cultures when clinically stable, and the presence of bacteria has been shown to be related to the degree of airflow obstruction and current cigarette smoking status[86,87].

A study done by Monso et al. and Rosell et al. studied stable and acute exacerbations among COPD patients and observed that lower airway bacterial colonizers with Stable COPD were similar to those bacteria detected during exacerbations[86,87]. *Klebsiella pneumoniae* isolated was significantly higher in AECOPD patients as compared to stable cases($P<0.001$)(Table 19).So in our study the *Klebsiella pneumoniae* isolated from Stable COPD patients could be airway colonizers as the isolation rate among AECOPD were high .The absence of symptoms in Stable COPD patients with lower airways colonized by potentially pathogenic organisms might be related to the bacterial load. Bacterial colonization might be due to failure to sterilise bronchial secretions after treatment of an episode of exacerbation which might lead to growth of microorganisms newer to lower airways without the symptoms of exacerbation[88,89,90].

The antibiogram performed for various isolates were analysed .The commonest Gram negative bacilli isolated from respiratory samples of AECOPD patients was *Klebsiella pneumoniae* showed Multi drug resistance (MDR) in 29.7% of patients .Resistance due to Cefotaxime Ciprofloxacin , and Cotrimoxazole in 62.2% , 43.3% and 43.3% respectively. Resistance to Imipenem was noted in

8.2% of isolates .(Table 22) Thus *Klebsiella pneumoniae* showed a significant level of resistance to third generation Cephalsporins and β lactamase inhibitor combinations . The prevalence of Carbapenemase production in *Klebsiella pneumoniae* was 28.5%. (Table :30)

This implies that *Klebsiella pneumoniae* was one of the important drug resistant pathogens isolated among AECOPD patients. The resistance to Cefotaxime ,Cotrimazole, Ciprofloxacin , (62.2% ,43.3%,and 43.3% respectively) and Imipenem 8.2% was higher in isolates from AECOPD patients than in Stable COPD patients. [Table:22,23]

Studies performed by Madhavi et al observed resistance of *Klebsiella pneumoniae* to Cefotaxime 62% ,Ciprofloxacin 42% and Cotrimoxazole 96% which was almost similar to our findings[(84)].

The other member of family Enterobacteriaceae isolated was *Klebsiella oxytoca* . Resistance to Cefotaxime 37.5%,Ciprofloxacin 12.5% and Cotrimoxazole 37.5% was also noted among *Klebsiella oxytoca* isolated with a MDR frequency of 12.5%. (Table 22)

The frequency of ESBL producers (Table :26) among Enterobacteriaceae was 40% among which 35.5% were *Klebsiella pneumoniae* isolates and 4.5% were *Klebsiella oxytoca* isolates. In a study done by Gerard rakesh et al.it was observed that 23.81% of *Klebsiella pneumoniae* were ESBL producers [(91)]. Study done by SMART

2007 stated ESBL rates in India for *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were, 69.4%, and 100%, respectively which was higher as compared to this study[102].

The most common non-fermenter isolated from the respiratory samples of AECOPD patients was *Pseudomonas aeruginosa* (Table:24),which showed 48.2% resistance to Ceftazidime. *Pseudomonas aeruginosa* had 3.7% Imipenem resistance. MDR strains were noted in 3.7% cases. Thus it showed a siginificant level of resistance to third generation Cephalosporin. This implies that *Pseudomonas aeruginosa* was also one of the important drug resistant pathogen isolated from cases of AECOPD.

This resistance pattern was similar to a study done by Chawla et al. who stated 60% resistance of *Pseudomonas aeruginosa* to third generation Cephalosporins[75].

This resistance pattern was similar to the studies done by Fluit *et al.* and Jones *et al.* who found that *Pseudomonas aeruginosa* had high level resistance to third generation Cephalosporins and also found that Piperacillin /tazobactam to be as potent as Amikacin, with resistance rates as low as 10–17%[93,94].

Non fermenter *Acinetobacter baumanii* isolates from AECOPD cases showed resistance to Ceftazidime 58.3% ,Ciprofloxacin 33.3% and Cotrimoxazole 33.3%. MDR(Multi drug resistant) strains were noted in 25% of *Acinetobacter baumanii* isolates.(Table 24) Thus it showed a significant level of resistance to third generation Cephalosporin. It showed 16.7% resistance to Imipenem .The proportion of *Acinetobacter baumanii* isolate producing Carbapenemase production was 14.3%

and 8% were Amp C producers (Table 29 ,30).This implies that *Acinetobacter baumanii* was also one of the important drug resistant pathogens isolated from cases of AECOPD.

This was in contrast to the study conducted in Asia pacific region by Wang huei Sheng et al. in 2013,where 15.3% of *Acinetobacter baumanii* isolates from lower respiratory tract infections were Amp C producers.

Thus in the present study ,among the mechanisms for developing resistance to third generation Cephalosporins, ESBL production was more commonly identified than Carbapenemase and Amp C production.No MBL producers were identified among Gram negative bacilli in the study.

Gram negative cocci (*Moraxella catarrhalis*) isolates from the respiratory samples of AECOPD patients showed 100% sensitivity to Ciprofloxacin, Erythromycin and Amoxycillin- Clavulanic acid combination.

Whereas in a study done by Karien et al have stated 2% of their Moraxella isolates were β lactamase producers.[8]

The most common gram positive isolates from respiratory samples in AECOPD patients in our study was *Staphylococcus aureus* (Table 14). The proportion of *Staphylococcus aureus* with Methicillin resistance was 37.5% . (Table 31). A study conducted by INSAR group stated that the prevalence of MRSA in India is about 40%[97]. This study statement correlates with this present study. In a study done by Gerard Rakesh et al. among AECOPD patients, the frequency of

Methicillin - Resistant *Staphylococcus aureus* was reported as 11.90% which was lesser as compared to our study observation. Chawla K et al .in their study observed that 50% of *Staphylococcus aureus* isolates were MRSA [(75)].All the MRSA isolates in our study was sensitive to Vancomycin.

All *Streptococcus pneumoniae* isolates from respiratory samples were 100% sensitive to Erythromycin, Cotrimoxazole, Ofloxacin, Tetracycline and Vancomycin. Two of the isolates showed resistance to Oxacillin by disc diffusion .(Table 20) The MIC to Penicillin which was determined by E strip showed intermediate resistance in 28.5%.(Table 32) .Thus there was a disparity of susceptibility between disc diffusion and MIC determination.

A study done by Goyal et al. in North India stated that 30 *Streptococcus pneumoniae isolates* were resistant to penicillin by Oxacillin disc diffusion method. Determination of MIC of these strains by broth dilution and E test revealed that 26 of isolates had intermediate resistance and only four isolates had complete resistance to penicillin .[(99)]This study also showed disparity of susceptibility between disc diffusion and MIC determination which was similar to this study.

Investigations of outbreaks by Center for Disease Control have revealed that Pneumococcal isolates resistant to penicillin in some areas of the United States is as high as 30%[(98)].

A high prevalence of Intermediate resistance to Penicillin (69.9%)among AECOPD patients has been noted by Fanny et al. which was quite higher as compared to our findings[92].

Study conducted by Iain B Gosbell *et al* stated that the prevalence of PRSP (Penicillin Resistant *Streptococcus pneumoniae)* was about 20%[100] . Emilio Perez – trallero et al. in their study stated that *Streptococcus pneumoniae* isolates obtained from AECOPD patients were more resistant to the antimicrobial agents generally used in the treatment of pneumococcal infections than those isolated from patients with pneumonia. This result was expected, as patients with COPD usually receive antimicrobial treatments because of frequent acute bacterial exacerbations and the association between antibiotic consumption and antimicrobial resistance has been demonstrated widely [101].

Though MDR pathogens were present in both moderate and severe type of COPD the frequency of MRSA and ESBL isolates were higher in Moderate AECOPD patients (66.7%, 66.7% , respectively) than in severe group(33.3%,33.3%). Penicillin resistant *Streptococcus pneumoniae* , Imipenem resistance, Carbapenamase producing isolates and Amp C producing strains were significantly higher in severe AECOPD patients than in moderate type (P value <0.001)(Table :34). MDR pathogens were present both in moderate and severe type of COPD.

Bacteremia among AECOPD patients was observed in 6% cases. (Table 10). In a study done by Laura solano et al., Spain ,bacteremia was noted in 10% of AECOPD patients admitted in Intensive care unit[77] .

The commonest pathogens isolated from blood were S*taphylococcus aureus* (44.4%) and *Klebsiella pneumoniae* (44.4%) followed by *Acinetobacter baumanii* (11.1%). Among *Staphylococcus aureus* 50% were MRSA. In *Klebsiella pneumoniae* ESBL production was observed in 75% cases. All *Acinetobacter baumanii* isolates showed 100% sensitivity to all drugs. **(**Table:25). No MRSA and ESBL producing blood stream isolates was observed in the study done by Laura Solano et. al.[77]

H. influenzae was not isolated in the present study. This could be due to prior antibiotic use or seasonal variations in causation. This is in concordance with previous studies conducted by Wilson *et al.,* in 1999 and Allegra et al in 1996[85,110].

No Fungal pathogens were isolated in this study which was corresponding to all other Indian studies [8,39].

SUMMARY

SUMMARY

This study conducted at the Institute of Microbiology, Madras Medical College and RGGGH in analyzing and comparing the etiologic agents of AECOPD and Stable COPD patients revealed the following findings.

- The majority number of AECOPD and Stable COPD patients were in 61-70 years age group.
- There was a predominance of males over females in both AECOPD (82.7% and 17.3%) and in stable COPD (84% and 16%) patients.
- Smoking was associated with AECOPD in76.6% of cases and 82% among COPD patients .Biogas inhalation was associated with AECOPD in 13.3% of cases and 14% among COPD cases. Smoking was the commonest risk factor in both the study groups.
- Chronic bronchitis was the most common condition in both stable (95.3%) and acute exacerbation(94%) of COPD.
- According to Anthosien criteria increased level of breathlessness was the commonest symptom in AECOPD (100%).Next most common symptom was increased in sputum volume(80%),and sputum purulence(72.6%)
- As per Anthosien criteria Type 1 exacerbation was the commonest type (72.6%) followed by Type3 exacerbation(20%).

- Complications were observed in (24%) patients among AECOPD patients among which Respiratory failure (8%) was the most common complication followed by Corpulmonale 11 (7.3%) and Bacteremia 9(6%).Stable COPD study group was not associated with any complication .
- Among AECOPD patients the most common type was Moderate type (57.3%) followed by Severe type (40%).Among Stable COPD patients the most common type was moderate type (82%) followed by Mild type (18%).
- The commonest sample for culture was Sputum sample (79.3%) followed by Endotracheal aspirate (8%).
- Bronchial wash and Endotracheal aspirate had the highest culture positivity rate of 100% and 91.6% respectively followed by sputum (64.7%).
- Among the respiratory samples 70.6% showed culture positivity.
- Pure Monobacterial type of isolates was 96.2%. Poly bacterial type of isolates were 3.8%.
- Among Gram positive organisms isolated from respiratory samples *Staphylococcus aureus* was the most commonly isolated in 15.6% cases and *Streptococcus pneumoniae* in 6.8% of patients.Among Gram negative organisms *Klebsiella pneumoniae* was the most commonly isolated organism (33.3%) followed by *Pseudomonas aeruginosa* (23.5%), Klebsiella oxytoca (6.8%), *Acinetobacter baumanii* (10.7%) and *Moraxella catarrhalis (*2.9%) .
- In moderate type of COPD patients *Klebsiella pneumoniae* contributed to 22.7% followed by *Staphylococcus aureus* 10.9% of infections.In severe type

Pseudomonas aeruginosa contributed to 20.9% followed by *Acinetobacter baumanii* (10.9%).

- Non fermenters were significantly isolated in Severe type as compared to the moderate type with a significant *P value of <0.001.*

- All Staphylococcus *aureus* isolates from AECOPD patients were 100% sensitive to Vancomycin.

- The frequency of MRSA in AECOPD patients was 37.5%.

- The frequency of Intermediate resistance to Penicillin in *Streptococcus pneumoniae* (PRSP) was 28.5%.

- The Isolation rate of ESBL producers were 40% among which 35.5% were *Klebsiella pneumoniae* isolates and 4.5% were *Klebsiella oxytoca* isolates.

- The prevalence of Carbapenemase production in *Klebsiella pneumoniae* was 28.5% and for *Acinetobacter baumanii* was 14.3% .

- The prevalence of Amp C producers in *Acinetobacter baumanii* was 8% .

- Presence of MRSA and ESBL isolates were higher in Moderate AECOPD patients (66.6%, 66.6% , r espectively) than in severe group(33.3%,33.3%).

- Penicillin resistant *Streptococcus pneumoniae* , Imipenem resistance, Carbapenamase producing isolates, Amp C producing strain were all more common in severe AECOPD patients (100%) than in moderate type which were statistically significant.

- The Polybacterial infection was found in three endotracheal aspirates and a single sputum sample among which the most common combined isolates were *Klebsiella pneumoniae and Pseudomonas aeruginosa.*

- In stable COPD patients *Klebsiella pneumoniae* was the only potential pathogen isolated in 18% of patients.

- 6% of the patients with AECOPD had Bacteremia.

- Bacteremia was observed in severe type of COPD only.

- No Fungal pathogens were isolated in this study .

CONCLUSION

CONCLUSION

Exacerbations punctuate the clinical course of COPD in many patients. Exacerbations, mostly of an infectious etiology, are a frequent cause of morbidity in COPD patients .So this study has been taken to analyse the bacterial and fungal profile with their sensitivity pattern.

In our study Bacterial infections are the most common reason for exacerbations, among which *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the commonest. Purulent sputum sample is a good and easy to obtain, non invasive sample that provides preliminary idea about the pathogens , thereby helping in selecting antibiotics for empirical antibiotic therapy as the culture positivity is high in these samples. Quantification of sputum sample is necessary to obtain the actual pathogen. As 6% of the patients showed Bacteremia it is necessary to perform blood culture in patients associated with fever. Few cases of polymicrobial infections was observed .

As *Klebsiella pneumoniae* were isolated from Stable COPD patients as well , these organisms could be airway colonizers as the isolation rate among AECOPD were also high .The absence of symptoms in Stable COPD patients with lower airways colonized by potentially pathogenic organisms might be related to lower bacterial load.

Antibiotics are important in treatment of AECOPD. Present study and few previous Indian studies have shown that bacterial pathogens responsible for AECOPD is different in our country from that of western countries and so is their

sensitivity pattern . *Pseudomonas aeruginosa* and *Acinetobacter baumanii* were significantly isolated in Severe type and *Klebsiella pneumoniae* from Moderate type . ESBL , Carbapenemase , AmpC, MRSA producers and Penicillin resistant *Streptococcus pneumoniae* strains were significantly isolated in Severe type of AECOPD patients in our study which increased the morbidity. MDR pathogens were present both in moderate and severe type of COPD. There was a disparity of susceptibility between disc diffusion and MIC determination in *Streptococcus pneumoniae* for Penicillin so it is always necessary to perfom MIC before reporting sensitivity pattern.

The choice of antibiotics depends on the local antibiotic policy and the pattern of local pathogens. Hence Periodic isolation and identification of resistant status of pathogens responsible for AECOPD will help us to formulate appropriate treatment protocol which will be of immense use in reducing mortality and morbidity besides reducing the volume of antibiotics and development of resistance to antibiotics.

APPENDIX

APPENDIX-I

ABBREVIATIONS

AECOPD	Acute exacerbation of Chronic obstructive pulmonary Disease
ATCC	American Type Culture Collections
CLSI	Clinical & Laboratory Standards Institute
COPD	Chronic obstructive pulmonary Disease
DDST	Double Disk Diffusion Synergy Test
ESBL	Extended Spectrum Beta Lactamases
FEV1	Forced expiratory volume
FVC	Forced vital capacity
GNB	Gram – Negative Bacilli
GPC	Gram – Positive Cocci
CAMHA	Cation adjusted Muller Hinton Agar
MBL	Metallo ß-Lactamases
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant Staphylococcus aureus
MSSA	Methicillin Sensitive Staphylococcus aureus
PCDDT	Phenotypic Confirmatory Disk Diffusion Test
RPMI 1640	Rose Parker Memorial Institute 1640
S.aureus	*Staphylococcus aureus*
MDR	Multi Drug Resistance

APPENDIX-II

A. STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g Methyl violet in 100ml absolute alcohol in 1 litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI(fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain

II. Lactophenol cotton blue stain

Lactic acid	20 ml
Phenol	20ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml

III. 10% KOH

Potassium hydroxide	10g
Glycerol	10ml
Distilled water	80ml

IV Acid fast stain

Basic fuchsin powder	5g
Phenol (crystalline)	25g
Alcohol(95% or 100% ethanol)	50ml
Distilled water	500ml

20% Sulphuric acid

Conc Sulphuric acid(98%)	250ml
Distilled water	1L

Methylene blue counterstain

1% Methylene blue

A. BROTH USED

1. Brain Heart Infusion broth:

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Calf brain, infusion (solids)	12.500
Beef heart, infusion (solids)	5.000
Dextrose	2.000
Sodium chloride	5.000
Disodium phosphate	2.500
Final pH (at 25°C)	7.4±0.2

37 grams of media is suspended in 1000 ml distilled water. Dispense into bottles or tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

2. Cation adjusted Mueller Hinton Broth:

Ingredients	Gms / Litre
Beef extract	3.000
Casein acid hydrolysate	17.500
Starch	1.500
Calcium	20-25 mg/l
Magnesium	10-12.5 mg/l
Final pH (at 25°C)	7.3±0.2

22 grams of media is suspended in 1000 ml of distilled water. Dissolve the medium completely. Dispense and sterilize by autoclaving at 115-121°C for 10 minutes. DO NOT OVERHEAT.

3. Trypticase soy broth

Ingredients	**Gms / Litre**
Pancreatic digest of casein	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000
Dextrose	2.500
Dibasic potassium phosphate	2.500
Final pH (at 25°C)	7.3±0.2

30 grams of media is suspended in 1000 ml of distilled water. Dissolve the medium completely. Dispense and sterilize by autoclaving at 115-121°C for 10 minutes. DO NOT OVERHEAT

B. MEDIA USED

1. Mac Conkey agar

Peptone	20g
S odium taurocholate	5 g
Distilled Water	1 ltr
Agar	20 g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

3. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

4. Sabouraud's dextrose agar

Dextrose	40 g
Peptone	10g
Agar	20g
Distilled water	1000ml
pH = 5.5	

5. Mueller- Hinton Agar

Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g
Agar	lOg
Distilled water	lltr
pH = 7.4	

Sterilise by autoclaving at 121°C for 20 mins

IDENTIFICATION

1.**Oxidase Reagent**

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2.Catalase

3% hydrogen peroxide

3.**Indole test**

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	lOg
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4.**Christensen's Urease test medium**

Peptone	lg
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	10ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr	
Agar	20 g	
Bromothymol blue	0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6.Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	lg
Lactose	10 g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
S odum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	l0mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8.Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube. Basal medium peptone water Sugar solutions:

Sugar	1ml
Dislilled water	100ml
pH = 7.6.	

9.Mannitol motility medium

Agar	5g
Peptone	lg
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	1000ml
pH	7.2

10.**Phenolphthalein diphosphate agar**

- Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4°C
- Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50°C and pour plates

- Grow the staphylococcus overnight at 37°C on the medium
- Invert the plate and pour a few drops of ammonia solution SG 0.88 into the lid
- Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades

11.2% Sodium deoxycholate solution:

Ingredients :

Sodium deoxycholate	2 gms
Distilled water	100ml

Dissolve 2 gms of deoxycholate in 100 ml of distilled water .Mix well.Store in a sterile containers.

12 .Decarboxylase media:

8a.Moller decarboxylase broth base:

Ingredients	gms/L
Peptone	5
Beef extract	5
Bromocresol purple	0.01
Cresol red	0.005
Glucose	0.5
Pyridoxal	0.005

Final pH 6

8b. Aminoacid:

Add 10 g of the levo form of the aminoacid for 1000ml.mix and dispense in sterile tubes.

9.Hugh & Leifson's Oxidation –Fermentation test:

Peptone	2g
Sodium chloride	5g
D-glucose	10g

Bromothymol blue	0.03g
Agar	3.0g
Dipotassium phosphate	0.30g
Distilled water	1L
pH =7.1	

Basal medium is autoclaved.1% of sterile sugar solutions is added to the basal medium. Dispense into sterile test tubes without slant.

10.Potassium nitrate broth

Potassium nitrate (KNO3)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

11. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

ANNEXURES

ANNEXURE-I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.V.R.YAMUNA DEVI,
Post Graduate in MD Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai-3.

Dear **Dr. V.R.YAMUNA DEVI.,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Bacterial and Fungal profile of Acute exacerbation of Chronic Obstructive Pulmonary Disease" "** No.17122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II

PROFORMA

- Name : IP no:
- Age: Ward:
- Sex:
- Occupation:
- Address:

Presenting complaints:

- High grade fever-
- Chills:
- Sweating:
- Dyspnea:
- Tachypnea:
- Chest pain:
- Cough :
- Sputum production:
- Nature of sputum,volume
- Hemoptysis:
- headaches
- loss of appetite:
- excessive fatigue:
- blueness of the skin (cyanosis):
- diarrhea:
- weight loss , Pedal edema
- Altered sensorium
- Musculoskeletal disorders(decrease lung func)

- Upper respiratory infection
- Gastroesophageal reflux
- aggravating and relieving factors

Past history:

previous episodes -asthma, bronchitis

TB ,

endocrine diseases,,

cardiac ,

lung ,

liver ,

renal disorders ,

chest injury,

hypertension,

Personal history:

- Alcohol intake:
- Cigarette smoking:
- Agriculture work:
- Exposure to chemicals occupationally

Family History

Immunization History

Drug History

Physical examination:

Chest X ray findings:

- lobar infiltrates or radiologic signs of pneumonia

Pulmonary function test:

Provisional diagnosis:

Laboratory evaluation:

Biochemical parameters:

- Plasma glucose levels
- Blood urea
- Creatinine
- Arterial blood gas analysis

Hematological investigations:

- TC
- DC
- Hb estimation
- ESR

Microbiological investigation:

Sample collected:

- Sputum:1)SPUTUM,2)INDUCED SPUTUM

  Bartlett score (for sputum sample):

- BRONCHIAL WASH
- ENDTRACHEAL ASPIRATE
- Blood

Direct examination:

Gram'stain:

AFB staining:

KOH mount:

Bacterial Culture:

- MAC
- BAP
- CAP

Fungal culture:

- SDA with antibiotics

Blood culture:

- Inoculated into Tryptic Soy broth with subculture onto
  - MAC
  - BAP
  - CAP

Isolate identified in sample:

Isolate identified in blood sample:

Antibacterial susceptibility pattern:

Antifungal susceptibility pattern:

ANNEXURE-III

CONSENT FORM

STUDY TITLE:

"Bacterial and fungal profile of acute exacerbation of Chronic obstructive pulmonary disease"

I……, hereby give consent to participate in the study conducted by Dr.V.R.Yamunadevi, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my Sputum/BAL fluid/and Blood samples for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal

Signature/ Thumb impression Place:

Of the patient/ relative Date:

Patient Name & Address:

Signature of the investigator:

Signature of the guide:

ANNEXURE-IV MASTER CHART-1

S. NO.	AGE	SEX		DIAGNOSIS SAMPLE	sample	ISOLATE1	ISOLATE 2	AK	GENT	CIP	COTRI	CEF	CZ	PT	IMI	PT	AMPI	AC	ERY	OFL OX	CHLO RA	TETRA	CEFO XITIN	OXA	OPTO	VAN C	PEN	BLOOD ISO	COUGH	FEVER	CREPTS/ WHEEZE	COMP	COMORBI D	SMOKER	BIOM ASS	TLC	NEUTR O	TYPE
95	80	M	121452	Emphysema	Induced sputum	Klebsiella pnuemonia		s	s	s	s	s	s	s													nil	purulent	nil	crepts			smoker		20500	92	mod
98	72	M	116328	chronic bronchitis	Sputum	Klebsiella oxytoca		s	s	s	s	s		s													nil	purulent	nil	crepts			smoker		15000	90	severe
113	55	M	57717	chronic bronchitis	Bronchial wash	Klebsiella pnuemonia		s	s	r	r	r	r	s(ESBL)													nil	purulent	nil	crepts			smoker		12000	89	severe
103	56	M	57661	chronic bronchitis	Endotracheal aspirate	Acinetobacter sp		r	r	r	r		r	r	r(MHT)64												nil	purulent	nil	crepts			smoker		14000	86	severe
1	45	M	120214	chronic	Sputum	NF																						mucoid	nil		cor		Smoker		7000	70	severe
4	52	M	124315	chronic bronchitis	Sputum	NF																						mucoid	nil				Smoker		6000	58	mild
11	60	M	2306	chronic bronchitis	Sputum	NF																						mucoid	nil				smoker		6000	68	mild
27	65	M	12148	chronic bronchitis	Sputum	Klebsiella pneumoniae		s	s	s	s	s	s	s													nil	mucopurulent	nil	crepts			smoker,alco		12200	89	mod
2	74	M	121450	chronic bronchitis	Sputum	Klebsiella pnuemoniae		s	s	s	s	r	r	s(ESBL)														purulent	nil	crepts			smoker		10000	89	mod
3	57	F	123183	chronic bronchitis	Sputum	Klebsiella pnuemoniae		s	s	r	s	s	s	s														purulent	yes	crepts				biogas	17500	87	mod
5	65	M	123735	chronic bronchitis	Sputum	Staphylococcus aureus		s		r								r	r				r(MRSA)			mic	r						smoker		9000	82	mod
								s		r								r	r				r(MRSA)			mic	r	Staphylococcus aureus	purulent	yes	crepts	corpulmonale		smoker,alcoholic		14000	90	severe
6	63	M	124451	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	r	r	s	s	s	s										r(MRSA)					nil	purulent	nil	crepts			smoker		10000	88	mod
7	65	M	1343	chronic bronchitis	Sputum	Staphylococcus aureus		s			s								r								r		purulent	yes	crepts		HT,DM	smoker		4000	83	mod
8	46	M	1477	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		s	s													nil	purulent	yes	crepts		DM	smoker,alcoholic		10000	78	severe
9	75	F	734	chronic bronchitis	Sputum	Staphylococcus aureus		s			s								s				s				s		purulent	nil	crepts		DM		biogas	6800	70	mod
10	72	M	1724	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	s	s	s	s													nil	purulent	nil	crepts			smoker,alcoholic		5600	80	mod
12	46	M	2318	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		s	s													nil	purulent	nil	crepts	corpulmonale		smoker		16000	76	severe
13	72	M	2052	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	r	r	r	s(ESBL)														purulent	nil	crepts			smoker,alcohol		7500	90	mod
14	85	M	770	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	s	s	s	s													nil	purulent	nil	crepts		anemia	smoker,alcoholic		8400	75	mod
15	61	M	4894	chronic bronchitis	Sputum	Klebsiella.pneumoniae		r	r	r	r	r	r	s(ESBL)	s	s						s					nil	purulent	nil	repts			smoker		8000	78	mod
16	45	M	5040	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	s	s	s	s													nil	purulent	nil	crepts			smoker		9000	75	mod
17	70	M	7596	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	r	r	s	s	s													nil	purulent	nil	creptss		sht,DM	smoker,alcoholic		9600	88	mod
18	58	M	7809	chronic bronchitis	Sputum	Klebsiella.pneumoniae			r	r	r	r	r	r	s							s					nil	mucopurulent	nil	wheeze		sht,	smoker,alcoholic		9400	70	severe
19	60	M	8010	chronic bronchitis	Sputum	NF																					nil	mucoid	nil	wheeze			smoker,alcohol		11400	70	mod
20	67	M	6639	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	r	r	r	s(ESBL)													nil	purulent	nil	crepts	corpulmonale		smoker		7700	85	severe
21	70	M	8740	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		s	s													nil	purulent	nil	crepts			smoker		11000	90	severe
22	53	M	8819	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	s	s	s	s													nil	purulent	yes	wheeze	cor pulmonale	SHT	smoker,alcohol		7600	68	mod
23	65	M	8384	chronic bronchitis	Sputum	Staphylococcus aureus		s		r	r						s		r				r(MRSA)					nil	mucopurulent	nil	crepts		SHT,DM	smoker,alcohol		5700	67	mod
24	75	F	11368	chronic bronchitis	Sputum	Acinetobacter sp		s	s	s	r		s	s	s												nil	purulent	nil	crepts				biogas	4300	60	severe
25	64	M	12123	chronic bronchitis	Sputum	NF																					nil	mucoid	nil	wheeze			smoker		6000	67	mild
26	55	M	12630	chronic bronchitis	Sputum	NF																					nil	mucoid	nil	wheeze			smoker		5800	62	mod
28	63	F	14414	chronic bronchitis	Sputum	NF																						mucoid						biogas	9100	60	mod

29	50	M	16438	chronic bronchitis	Sputum	Pseudomonas aeruginosa		r	r	r	r		s	s														nil	purulent	ye	crepts			smoker		11400	74	severe
30	75	M	15827	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	r	r		s	s														nil	purulent	yes	crepts		DM	smoker		15000	89	severe
31	50	M	17147	chronic bronchitis	Bronchial wash	Acinetobacter sp		s	s	s	r		s	s	s													nil	purulent	yes	crepts	corpulmonale	DM	smoker		11000	68	severe
32	83	M	11342	chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze		SHT	smoker		17600	90	mod
33	55	M	12133	chronic bronchitis	Sputum	Klebsiella.pneumoniae		s	s	s	s	r	r	s(ESBL)														nil	mucopurulent	nil	crepts			smoker		18000	93	mod
34	86	M	14131	chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		8000	70	mod
35	75	M	21199	chronic bronchitis	Sputum	NF																							mucoid					smoker		5500	65	mod
36	70	M	19788	chronic bronchitis	Sputum	Acinetobacter sp		s	s	s	r		r	s	s	s													purulent	yes	crepts		Sht,anem	smoker		15000	78	sev
								s	s	s	r	r	r	s	s	s												acinetobacter sp											
37	60	M	20839	Chronic bronchitis	Sputum	Staphylococcus aureus		s									s											nil	purulent	nil	crepts			smoker		4500	65	mod
38	68	M	21357	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		5100	84	mod
39	55	M	22239	Chronic bronchitis	Sputum	Staphylococcus aureus		s		s	s								s									nil	purulent	nil	wheeze			smoker		6000	70	mod
40	70	M	24206	Chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		r	s(ESBL)	s	s(ESBL)												nil	purulent	nil	crepts	corpulmonale		smoker		10000	70	sev
41	90	M	25375	Chronic bronchitis	endotracheal aspirate	Staphylococcus aureus		s		s	s						s		s								same	staphylococcus aureus	purulent	yes	crepts	resp failure 2		smoker		11000	86	sev
								s		s	s						s		s									staphylococcus aureus										
42	55	M	25087	Chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	r	r	r	r	s(ESBL)														nil	purulent	nil	crepts			smoker		17100	87	mod
43	66	F	26321	Chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	r	r	r	r	s(ESBL)														nil	purulent	nil	crepts				biogas	15500	87	mod
44	50	M	37810	Chronic bronchitis	Sputum	Acinetobacter sp		s	s	s	r		s	s	s													nil	mucopurulent	nil	crepts	resp failure 1		aluminium worker		6900	75	sev
45	56	F	42177	Chronic bronchitis	Sputum	Klebsiella pnuemonia		s	r	r	r	r	r	r	s													nil	purulent	nil	crepts				biogas	10000	70	mod
46	62	M	47384	Chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	s	s	s	s														nil	purulent	nil	crepts			smoker		14000	77	mod
47	64	M	51771	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		6000	70	mod
48	45	M	52096	Emphysema	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		9000	64	mod
49	75	M	52107	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		6000	70	mod
50	73	M	51977	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		8000	85	mod
51	46	M	52071	Chronic bronchitis	Sputum	NG																						nil	mucoid	nil	wheeze	resp failure 2		smoker		8000	70	sev
52	49	M	57086	Chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	s	s	s	s														nil	purulent	nil	crepts			smoker		8000	80	mod
53	60	M	42828	Chronic bronchitis	Sputum	Klebsiella oxytoca		s	s	s	r	r		s(ESBL)														nil	purulent	nil	crepts	resp failure 1		smoker		11000	70	mod
54	77	M	41327	Chronic bronchitis	Bronchial wash	Acinetobacter sp		s	r	s	r		r	s(ESBL)	s													nil	mucopurulent	nil	wheeze	corpulmonale		smoker		10000	78	sev
55	60	F	53812	Chronic bronchitis	Sputum	NG																						nil	mucoid	nil	crepts				biogas	6300	80	mod
56	76	M	53595	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	crepts			smoker		7000	65	mod
57	45	M	53355	Chronic bronchitis	Endotracheal aspirate	Klebsiellaoxytoca		s	r	r	s	r		r	r(MBL)													nil	purulent	nil	crepts			smoker		14000	80	sev
							Acinetobacter sp	s	r	r	r	r	r	r	r(MBL)																							
58	64	M	544411	Chronic bronchitis	sputum	NF																						nil	mucoid	nil	wheeze			smoker		7000	65	mod
59	67	M	85632	chronic bronchitis	Sputum	Klebsiellapnuemonia		s	s	s	s	s	s	s	s													nil	purulent	nil	crepts			smoker		10000	88	mod

60	47	M	55597	chronic bronchitis	Bronchial wash	Pseudomonas aeruginosa		s	s	s	r		s	s(ESBL)														nil	purulent	nil	crepts	resp failure 1		smoker		12000	90	sev
61	61	M	52590	chronic bronchitis	Bronchial wash	Pseudomonas aeruginosa		s	s	s	r		s	s														nil	purulent	nil	crepts	resp failure 1		smoker		11000	90	sev
62	58	M	55990	chronic bronchitis	Endotrachial aspirate	Pseudomonas aeruginosa		r	r	r	r		r	r	s	r												nil	purulent	yes	crepts	resp failure 2		smoker		8100	89	sev
63	60	M	57650	chronic bronchitis	Endotrachial aspirate	NF																						nil	mucoid	yes	crepts	resp failure 1		smoker		9000	78	sev
64	55	M	60711	chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	s	r	r	s(ESBL)														nil	purulent	nil	crepts	renal failure		smoker		10000	70	mod
65	55	M	57956	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	r	r	r		r	s(ESBL)	s													nil	purulent	nil	crepts			smoker		9000	75	mod
66	75	F	52539	chronic bronchitis	Endotrachial aspirate	Klebsiella pnuemonia		r	r	r	r	r		r	s														purulent	yes	crepts	resp failure 2death			biogas	12000	90	sev
								r	r	r	r	r		r	s	s												Klebsiella pnuemonia										
67	70	F	61563	Chronic bronchitis	Sputum	Psuedomonas aerubinosa		s	r	s	r		r	r	r(MBL)	r												nil	purulent	nil	crepts				Biogas	10000	85	sev
68	62	F	61539	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze				Biogas	7000	68	mod
69	80	M	62312	Chronic bronchitis	Sputum	Psuedomonas aerubinosa		s	s	s	r		r	s(ESBL)	s													nil	purulent	nil	crepts	resp failure 1		smoker		12000	88	sev
70	56	M	57661	Chronic bronchitis	Endotracheal aspirate	Klebsiella pnuemonia		r	r	r	r	r	r	r	s(mic)													nil	purulent	nil	crepts	resp failure 1		smoker		12000	90	sev
							Psuedomonas aeruginosa	r	r	s	r		r	s(ESBL)														nil	mucopurulent	nil	crepts	resp failure 1		smoker		11000	88	sev
71	61	M	52692	Chronic bronchitis	Sputum	Psuedomonas aerubinosa		s	s	s	r		r	s(ESBL)	s													nil	purulent	nil	crepts			smoker		11500	90	mod
72	57	M	95431	chronic bronchitis	Sputum	NF																						nil	purulent	nil	crepts			smoker		6000	65	mod
73	56	M	95451	chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	r	s		s													same	Klebsiella pnuemoniae	purulent	yes	crepts			smoker		10000	88	mod
74	58	M	98074	chronic bronchitis	Sputum	NF																						nil	mucopurulent	nil	wheeze			carpenter		9000	85	mod
75	58	F	98154	chronic	Sputum	Psuedomonas		s	s	s	r		s	s														nil	purulent	nil	crepts				Bioga	12000	90	mod
76	56	M	99899	chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	r	r	r		s(ESBL)													same	Klebsiella pnuemoniae	purulent	yes	crepts			smoker		13900	91	mod
77	53	M	99716	chronic bronchitis	Sputum	Streptococcus pneumoniae													r				s			s		nil	purulent	nil	crepts		SHT	smoker		11900	78	mod
78	62	M	55005	chronic bronchitis	Sputum	Staphylococcus aureus		s			r	r																nil	purulent	nil	crepts		RHD	smoker		11000	70	mod
79	74	M	101322	chronic bronchitis	Sputum	Streptococcus pneumoniae																	s					nil	purulent	nil	crepts			smoker		10000	89	mod
80	60	M	102660	chronic bronchitis	Sputum	Moraxella catarrhalis				s	r							s	s			r						nil	mucopurulent	nil	crepts			smoker		11000	90	mod
81	74	M	103003	chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	r	r		s(ESBL)													same	Klebsiella pnuemoniae	purulent	nil	wheeze		DM	smoker		14000	98	sev
							Psuedomonas aeruginosa	s	s	s	r		r	s(ESBL)																								
82	45	M	106324	Chronic bronchitis	Sputum	NF																						nil	mucopurulent	yes	wheeze			smoker		9000	78	mod
83	62	M	107563	chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	s	r	r	s(ESBL)														nil	purulent	nil	crepts			smoker		9000	78	severe
84	75	M	107080	chronic bronchitis	Sputum	NF																						nil	mucoid	Yes	wheeze		SHT,AS	smoker		6400	91	severe
85	70	M	107152	chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		6500	88	mod
86	65	M	167944	chronic bronchitis	Sputum	Streptococcus pneumoniae																	s					nil	mucopurulent	nil	wheeze			smoker	alcoholic	7900	90	severe

87	58	M	108901	chronic bronchitis	Sputum	Staphylococcus aureus		s		r	r													s(MSSA)				s	nil	purulent	nil	wheeze			smoker	alcoholic	11300	85	mod
88	59	M	109968	chronic bronchitis	Sputum	NF																							nil	mucoid	nil	wheeze			smoker		10800	84	mod
89	65	M	100070	chronic bronchitis	Sputum	Staphylococcus aureus		s												s				r(MRSA)				r	nil	mucopurulent	nil	crepts			smoker		16800	80	mod
90	65	M	111193	chronic bronchitis	Sputum	Staphylococcus aureus		s		s										s				s(MSSA)				s	nil	mucopurulent	nil	wheeze			smoker	Biogas	18400	75	mod
91	40	F	110822	chronic	Sputum	Streptococcus																			s				nil	mucopuru	nil	wheeze				Bioga	16000	85	severe
92	55	M	110153	chronic bronchitis	Sputum	NF																							nil	mucoid	nil	wheeze			smoker		7000	70	mod
93	75	F	111090	chronic bronchitis	Sputum	Streptococcus pneumoniae																			s				nil	mucopurulent	nil	crepts		DM,SHT,RF		Biogas	10500	80	severe
94	53	F	113752	Emphysema	Induced sputum	Klebsiella pnuemonia		s	s	r	s	r	r	s(ESBL)															nil	purulent	nil	crepts				Biogas	15000	88	mod
96	55	M	113275	chronic bronchitis	Sputum	Psuedomonas aerubinosa		s	r	s	r		s	s															nil	purulent	nil	crepts			smoker		5800	60	severe
97	60	M	11547	chronic bronchitis	Endotracheal aspirate	Streptococcus pneumoniae																			r				nil	purulent	nil	crepts		CAD	smoker		17600	91	severe
99	83	F	118485	chronic	Sputum	Acinetobacter sp		r	r	r			r	r	s	s													nil	purulent	nil	wheeze		SHT		Bioga	12300	80	severe
100	65	M	110304	chronic bronchitis	Sputum	Klebsiella pnuemonia		s	s	s	s	s	s	s															nil	purulent	nil	crepts			smoker	alcoholic	15000	92	mod
101	48	M	119908	chronic bronchitis	Sputum	NF																							nil	mucoid	nil	wheeze			smoker		15600	88	mod
102	62	M	119801	chronic bronchitis	Sputum	Staphylococcus aureus		s		s										r				r(MRSA)				(rsame)	staphylococcus aureus	purulent	yes	crepts			smoker	alcoholic	15000	90	severe
104	85	F	64366	chronic bronchitis	Sputum	NF																							nil	mucoid	nil	wheeze				Biogas	8000	68	mod
105	63	F	64425	chronic bronchitis	Sputum	NF																							nil	mucoid	nil	wheeze				Biogas	7000	68	mod
106	86	M	64179	chronic bronchitis	Sputum	Staphylococcus aureus		s		r										s				s(MSSA)					nil	purulent	nil	crepts			smoker		11000	75	mod
107	52	F	53481	chronic bronchitis	Sputum	Klebsiella oxytoca		s	s	s	r	r		s(ESBL)															nil	purulent	nil	crepts				Biogas	12000	88	mod
108	55	F	62902	chronic bronchitis	Sputum	NF																							nil	purulent	nil	crepts				Biogas	6000	68	mod
109	60	M	62208	chronic bronchitis	Sputum	NF																								mucoid	nil	crepts			smoker		7000	70	mod
110	69	M	65502	chronic bronchitis	Sputum	NF																								mucoid	nil	crepts			smoker		8000	79	mod
111	42	M	64155	chronic bronchitis	Sputum	NF																								mucoid	nil	crepts			smoker		9000	78	mod
112	70	F	65683	chronic bronchitis	Sputum	NF																								mucoid	nil	crepts				Biogas	7500	80	mod
114	55	M		chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		s	s																purulent	nil	crepts			smoker		11000	90	severe
115	65	M	625683	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		s	s															nil	purulent	nil	crepts	respiratory failure 2		smoker		12000	90	severe
116	75	M	246392	chronic bronchitis	Sputum	Pseudomonas aeruginosa		s	s	s	r		s	s															nil	purulent	nil	crepts	respiratory failure 2		smoker		11000	80	severe
117	80	M	219286	chronic bronchitis	Induced sputum	Psuedomonas aerubinosa		s	s	s	r		r	s(ESBL)	s														nil	purulent	nil	crepts	respiratory failure 2		smoker		10000	89	severe
118	82	M	222392	chronic bronchitis	Induced sputum	Klebsiella oxytoca		s	s	s	s	s		s															nil	purulent	nil	crepts		DM,	smoker		11000	88	mod
119	69	F	234123	chronic bronchitis	Bronchial wash	Klebsiella pnuemonia		s	s	s	s	r	r	s(ESBL)															nil	purulent	nil	crepts		PHT		Biogas	13000	92	severe
120	75	F	216743	chronic bronchitis	Bronchial wash	Acinetobacter sp		s	s	s	r		s	s	s														nil	purulent	nil	crepts	respiratory failure 2			Biogas	10500	90	severe
121	88	M	298214	chronic bronchitis	Sputum	Staphylococcus aureus		s		s	s						r		s					r(MRSA)				nil	purulent	nil	crepts		DM	smoker		11500	92	severe
122	56	M	256732	Chronic bronchitis	Endotracheal aspirate	Klebsiella pnuemonia		r	r	r	r	r	r	r	r(mic)														nil	purulent	nil	crepts	respiratory failure 2		smoker		12800	90	severe
123	75	F	209872	chronic bronchitis	Sputum	Streptococcus pneumoniae																			r				nil	mucopurulent	nil	crepts		DM,SHT,RF		Biogas	10500	80	severe

124	53	F	213456	Emphysema	Induced sputum	Klebsiella pnuemonia		s	s	r	s	r	r	s(ESBL)														nil	purulent	nil	crepts				Biogas	15000	88	mod
125	58	F	243158	chronic bronchitis	Sputum	Psuedomonas aerubinosa		s	s	s	r		s	s														nil	purulent	nil	crepts				Biogas	12000	90	mod
126	48	M	267821	chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		15600	88	mod
127	75	M	259014	chronic bronchitis	Sputum	NF																						nil	mucoid	Yes	wheeze		SHT,AS	smoker		6400	76	mod
128	45	M	267451	Emphysema	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		9000	64	mod
129	68	M	221357	Chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		5100	84	mod
130	45	M	286253	chronic bronchitis	Sputum	NF																						nil	mucoid	nil	wheeze			smoker		6500	75	mod
131	85	M	278523	chronic bronchitis	Endotracheal aspirate	Klebsiella pnuemonia		s	s	s	s	r	r	s(ESBL)														nil	purulent	yes	crepts	respiratory failure		smoker		13000	92	severe
							Psuedomonas aeruginosa	s	s	s	r		r	s(ESBL)																							
132	82	M	256321	Chronic bronchitis	Induced sputum	Acinetobacter sp		r	r	r	r		r	s(ESBL)	s								s					nil	purulent	nil	crepts	Corpulmonale		smoker		12000	90	severe
133	68	M	209768	chronic bronchitis	Bronchial wash	Acinetobacter sp		s	s	s	r		r	s(ESBL)	s													nil	purulent	nil	crepts	Corpulmonale		smoker		11000	89	severe
134	65	M	231567	chronic bronchitis	Sputum	Moraxella catarrhalis				s	s							s	s			s						nil	purulent	nil	crepts			smoker		9000	78	mod
135	69	M	289765	Chronic bronchitis	Sputum	Moraxella catarrhalis				s	s							s	s			s						nil	purulent	nil	crepts			smoker		9500	78	mod
136	79	M	217890	Emphysema	Induced sputum	Pseudomonas aeruginosa		s	s	s	r		s	s														nil	purulent	nil	crepts			smoker		11000	90	severe
137	80	M	227890	Chronic bronchitis	Bronchial wash	Pseudomonas aeruginosa		s	s	s	r		r	r	s	r												nil	purulent	nil	crepts	respiratory failure 1		smoker		12000	90	severe
138	65	M	234156	Chronic bronchitis	Sputum	Klebsiella oxytoca		s	s	s	s	s		s														nil	purulent	nil	crepts			smoker		10000	88	mod
139	60	M	219023	Chronic bronchitis	Induced sputum	Staphylococcus aureus		s		s	s								s			s						nil	mucopurulent	nil	crepts			smoker		11000	89	mild
140	65	M	235678	Emphysema	Sputum	Pseudomonas aeruginosa		s	s	s			r	s(ESBL)	s													nil	purulent	nil	crepts			smoker		10000	90	severe
141	70	M	253142	Chronic bronchitis	Bronchial wash	Klebsiella pnuemonia		s	s	s	s	r	r	r	r(MIC)256													nil	purulent	nil	crepts	respiratory failure 1		smoker		11000	92	severe
142	75	M	289731	Chronic bronchitis	Endotracheal aspirate	Klebsiella pnuemonia		s	s	r	r	r	r	r	r(MIC)													nil	purulent	nil	crepts	respiratory failure 2		smoker	alcoholic	13000	90	severe
143	55	M	302134	Chronic bronchitis	Sputum	Klebsiella oxytoca		s	s	s	s	s		s														nil	purulent	nil	crepts			smoker		10000	88	mod
144	62	F	334252	Chronic bronchitis	Sputum	Klebsiella oxytoca		s	s	s	r	s		s														nil	purulent	nil	crepts				Biogas	9000	80	mod
145	78	M	312678	Chronic bronchitis	Endotracheal aspirate	Pseudomonas aeruginosa		s	s	s			r	s(ESBL)	s													nil	purulent	nil	crepts	respiratory failure 2		smoker		13000	88	severe
146	88	M	321456	Chronic bronchitis	Induced sputum	Ng																						nil	mucoid	nil	wheeze	sever PHT		smoker		8500	72	severe
147	82	M	302435	Chronic bronchitis	Sputum	Ng																						nil	mucoid	nil	wheeze			smoker		9000	75	mod
148	80	M	316789	Chronic bronchitis	Sputum	Acinetobacter sp		s	s	s			s	s	s													nil	mucoid	nil	crepts			smoker		12500	92	severe
149	55	M	321564	Chronic bronchitis	Sputum	NG																						nil	mucoid	nil	wheeze			smoker		7500	70	mild
150	68	M	346179	Chronic bronchitis	Sputum	Staphylococcus aureus		s		s	s						s		s			s						nil	purulent	nil	crepts			smoker		12000	90	mod

MASTER CHART-2

S. NO.	AGE	SEX		DIAGNOSIS SAMPLE	ISOLATE	AK	GENT	CIP	COTRI	CEF	CZ	CS	COMOR BID	SMOKER	BIOMASS	TYPE	ADMISSION	OCCU
1	51	M	OP 772/14	Chronic bronchitis	NF									smoker		mod	2	
2	75	M	OP	chronic bronchitis	Klebsiella pnuemonia	S	S	S	R	S		S		non smoker		mod	no	weaver
3	32	M	OP	chronic bronchitis	NF									smoker		mod	no	welder
4	60	M	OP	chronic bronchitis	NF									smoker		mod	1	business
5	34	M	OP	Emphysema	NF									non smoker		mod		a1 antitrypsin def
6	53	M	OP	chronic bronchitis	NF									Smoker		mod		ironing
7	50	M	OP	chronic bronchitis	NF									Smoker		mod		business
8	62	M	OP	Emphysema	Klebsiella pneumoniae	S	S	S	S	S		S		smoker		mod		business
9	73	M	OP	chronic bronchitis	NF									smoker		mod		painting
10	58	M	OP	chronic bronchitis	NF									Smoker		mod		hotel supplier
11	62	M	OP	chronic bronchitis	Klebsiella pnuemonia	S	S	S	S	S		S		Smoker		mild		security
12	62	M	OP	Chronic bronchitis	NF									smoker		mod		
13	60	M	OP	chronic bronchitis										non smoker		mod		
14	69	M	OP	chronic bronchitis	Klebsiella pnuemonia	S	S	S	S	S		S		smoker		mild	1	
15	45	F	OP	chronic bronchitis	NF									non smoker	BIOMASS	mild	no	labour
16	50	M	OP	chronic bronchitis	NF									smoker		mild		
17	64	M	OP	chronic bronchitis	NF									smoker		mod	2	
18	51	M	OP	chronic bronchitis	NF									smoker		mod		
19	55	M	OP	chronic bronchitis	NF									smoker		mild	1	
20	61	M	OP	chronic bronchitis	NF									smoker		mod		
21	65	M	OP	chronic bronchitis	NF									smoker		mod		

22	56	M	OP	chronic bronchitis	NF									smoker		mod		
23	65	M	OP	chronic bronchitis	Klebsiella pneumoniae	S	S	S	S	S		S		smoker		mod		
24	54	M	OP	chronic bronchitis	NF									smoker		mod		
25	60	M	OP	chronic bronchitis	NF									smoker		mod		
26	57	M	OP	chronic bronchitis	NF									smoker		mod		
27	62	F	OP	chronic bronchitis											BIOMASS	mild		
28	53	F	OP	chronic bronchitis	Klebsiella pneumoniae	S	S	S	S	S		S			BIOMASS	mild		
29	65	M	OP	chronic bronchitis	NF									smoker		mod		
30	68	F	OP	chronic bronchitis	Nf										BIOMASS	mod		
31	64	M	OP	chronic bronchitis	NF									SMOKER		mod		
32	55	M	OP	chronic bronchitis	NF									smoker		mod		
33	63	F	OP	chronic bronchitis	NF										BIOMASS	mod		
34	50	M	OP	chronic bronchitis	NF									smoker		mild		
35	65	M	OP	chronic bronchitis	NF									smoker		mod		
36	50	M	OP	chronic bronchitis	NF									smoker		mod		
37	63	M	OP	chronic bronchitis	NF									smoker		mod		
38	55	M	OP	chronic bronchitis	Klebsiella pneumoniae	S	S	S	S	S		S		smoker		mod		
39	71	M	OP	chronic bronchitis	NF									smoker		mod		
40	70	M	OP	chronic bronchitis	NF									SMOKER		mod		
41	65	M	OP	chronic bronchitis	NF									smoker		mod		
42	62	F	OP	Chronic bronchitis	NF										Biogas	mod		
43	60	M	OP	Chronic bronchitis										smoker		mod		

44	68	M	OP	Chronic bronchitis	NF									smoker		mild		
45	55	M	OP	Chronic bronchitis	NF									smoker		mod		
46	70	M	OP	Chronic bronchitis	NF									smoker		mod		
47	78	M	OP	Chronic bronchitis	NF									smoker		mod		
48	55	F	OP	Chronic bronchitis	NF										Biogas	mod		
49	55	M	OP	Chronic bronchitis	Klebsiella pnuemonia	S	S	S	R	S		S		smoker		mod		
50	66	F	OP	Chronic bronchitis	Klebsiella pnuemonia	S	S	S	S	S		S			Biogas	mod		

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